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BIOLOGICAL BULLETIN

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Marine Biological Laboratory

WOODS HOLE, MASS.

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CONTENTS OF VOLUME XXIII.

No. 1. June, 1912.

Child, C. M. *The Process of Reproduction in Organisms* 1

Dederer, Pauline H. *Preliminary Note on Gametogenesis in Philosamia cynthia* 40

Kepner, Wm. A., and Taliaferro, W. H. *Sensory Epithelium of Pharynx and Ciliated Pits of Microstoma caudatum* 42

No. 2. July, 1912.

Shelford, Victor E. *Ecological Succession. IV. Vegetation and the Control of Land Animal Communities* 59

Newman, H. H. *The Ovum of the Nine-banded Armadillo. Growth of the Ovocytes, Maturation and Fertilization* 100

No. 3. August, 1912.

Boring, Alice M. *The Interstitial Cells and the Supposed Internal Secretion of the Chicken Testis* 141

Peebles, Florence. *Regeneration and Regulation in Paramecium caudatum* 154

Jackson, C. M. *On the Recognition of Sex Through External Characters in the Young Rat* 171

Morgan, T. H., and Lynch, Clara J. *The Linkage of Two Factors in Drosophila that are not Sex-linked* 174

Dexter, John S. *On Coupling of Certain Sex-linked Characters in Drosophila* 183

Watters, Florence A. *Size Relationships between Conjugants and Non-conjugants in Blepharisma undulans* 195

No. 4. September, 1912.

Glaser, R. W. *A Contribution to Our Knowledge of the Function of the Œnocytes of Insects* 213

Harvey, B. C. H., and Bensley, R. R. *Upon the Formation of Hydrochloric Acid in the Foveolæ and on the Surface of the Gastric Mucous Membrane and the Non-acid Character of the Contents of Gland Cells and Lumina* 225

Weschè, W. *The Phylogeny of the Nemocera, with Notes on the Leg Bristles, Hairs and Certain Mouth Glands of Diptera* ... 250

No. 5. October, 1912.

Patterson, J. T., and Wieman, H. L. *The Uterine Spindle of the Polyclad Planocera inquilina* 271

Strong, R. M. *Results of Hybridizing Ring-Doves, Including Sex-linked Inheritance* .... 293 ✓

Whitney, D. D. *Weak Parthenogenetic Races of Hydatina senta Subjected to a Varied Environment* 321

No. 6. November, 1912.

Shelford, Victor E. *Ecological Succession. V. Aspects of Physiological Classification* 331

Turner, C. H. *An Experimental Investigation of an Apparent Reversal of the Responses to Light of the Roach (Periplaneta orientalis L.)* 371

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BIOLOGICAL BULLETIN

THE PROCESS OF REPRODUCTION IN ORGANISMS.

C. M. CHILD.

The present trend of certain lines of biological thought and investigation is toward the conclusion that the process of sexual reproduction in organisms is something unique and associated with a peculiar substance, the germ plasm. This substance is often regarded as self perpetuating, as "continuous" and as independent, except for nutrition, of the organism or soma in which it lives as a sort of parasite and it is the repository of all heritable capacities or characteristics. According to this view the problem of heredity is a problem of the germ plasm and of the germ plasm alone. We are all familiar with the attempts which have been made to interpret the phenomena of asexual reproduction and of regulation with the aid of this hypothesis and the difficulties which these interpretations have encountered are matters of biological history. Moreover, about this hypothesis has grown up a great mass of involved speculation which to many constitutes the foundation of modern biology and which is often accepted as fact rather than hypothesis.

My own lines of investigation and thought have led me to the belief that this germ plasm hypothesis and the subsidiary hypotheses which have grown up about it are not only unnecessary and constitute an impediment to biological thought which has retarded its progress in recent years to a very appreciable extent, but furthermore, that they are not in full accord with observed facts and can be maintained only so long as we ignore the facts. The present paper is an attempt to show that a logical and far more simple theory of reproduction and inheritance is possible on a basis which does not involve so many assumptions of doubtful value and which does agree with the facts of observation.

I. The Reconstitution of Isolated Pieces of Organisms.

If we isolate physically a piece of one of the simpler organisms capable of regulation, *e. g.*, *Hydra*, *Planaria*, we know that within certain limits of size and other conditions the isolated piece reconstitutes itself into a new individual which possesses the essential characteristics of the species. Here then is a case of reproduction induced experimentally.

But what initiates the process of reconstitution? Why does the part become a whole? Most authorities are agreed at present that the isolation of the part is the essential factor in initiation of the process of reconstitution. Before isolation the part in question was in physiological correlation with other parts, *i. e.*, the processes going on in them influenced it in one way or another, affecting the rate, the character, the localization or the sequence of the processes occurring in it. With the act of isolation all these influences cease. What is the result? We see first that the piece or some part of it undergoes a process of dedifferentiation. It may lose to a greater or less extent the structural features which were characteristic of it as a part or it may give rise to embryonic tissue at the cut surface. Sooner or later this process of dedifferentiation is succeeded by a process of redifferentiation and the part gradually becomes a new whole organism.

In this process it is evident, first that the dedifferentiation is the result of the isolation. So long as the piece was in correlation with other parts it remained a part. After isolation it lost the features which characterized it as a part. Secondly, having lost these features to a greater or less extent it began to behave in the manner characteristic of the protoplasm of the species to which it belongs, *i. e.*, it began to develop into a new individual with the specific characteristics. The process of dedifferentiation is not an assumption but an observed fact. It has long been known that in plants many cells are capable of undergoing dedifferentiation. In the lower animals various cells which are certainly not morphologically undifferentiated are capable of becoming embryonic in character and of giving rise, *e. g.*, in regulation, to various parts, and, in nature, to germ cells. These cells are often regarded as indifferent or undifferentiated but I believe that the facts do not warrant such a designation. The cells are in many cases functional parts of the organism and even their histological characteristics indicate that they are by no means indifferent or reserve cells. Cases in point are the parenchyma of the flat worms and the peritoneal epithelium of polychætes. Apparently in many cases such cells have been called indifferent not because of their embryonic appearance, but because they are capable of forming other parts or of giving rise to germ cells. If we are to hold to the morphological criterion of differentiation then these cells certainly undergo dedifferentiation before they redifferentiate.

The assumption of the existence of accessory germ plasm to account for such cases is entirely unnecessary and superfluous. If protoplasm is a physico-chemical substance why is it necessary to assume the continuous existence of a given specific constitution. Other substances in nature are capable of changing their constitutions in one direction or another under certain conditions, while under others they may return to their original constitution. Some of the most fundamental laws of physics and chemistry are based upon this fact. Moreover, we know that in the metabolic processes of organisms substances may be built up under certain conditions and decomposed under others. What conceivable reason have we for assuming before we have tested all possibilities that these same laws do not hold good for what we are accustomed to call germ plasm?

In short, the evidence of our senses teaches us that in the isolated pieces capable of regulation something identical with or approaching more or less closely to the so-called germ plasm of the species appears *de novo* in consequence of the isolation and that this substance reacts in a definite specific manner essentially similar to the reaction of other isolated masses of the protoplasm of the species. Here then is a case of reproduction for which the assumption of continuity of the germ plasm is purely gratuitous. Whenever in such organisms the conditions which induce differentiation disappear the differentiation disappears to a greater or less degree, in part through the destruction of the more highly differentiated cells and in part through the dedifferentiation of the less highly differentiated cells which are then capable of undergoing redifferentiation. In other words, the cells which are capable of regulation approach or return to the general type of reaction characteristic of the specific protoplasm.

II. Rejuvenescence in Reconstitution.

The new whole which arises from the experimentally isolated part may be much smaller than the whole of which it originally formed a part. But the question at once arises, is it younger? Has the process of dedifferentiation carried it back again toward the beginning of the developmental cycle? I have recently shown (Child, '11*b*) that for *Planaria* this question must be answered in the affirmative and I have further evidence of the same kind for other forms. The new individual resulting from the regulation of a piece is in all respects younger than the animal of which it formed a part. Its rate of metabolism is higher, it is capable of renewed growth and grows at the same rate as a young animal: in every respect it has undergone a process of rejuvenescence. Moreover, the degree of rejuvenescence is in general proportional to the degree of reorganization in the piece, *i. e.*, the degree to which the old structure has disappeared and new structure developed. This process of rejuvenescence involves not only the portions directly concerned in regeneration of lost parts but to a greater or less extent the other portions of the body as well. For example, the removal of the posterior portion of the body of *Planaria* brings about rejuvenescence not only in the posterior region of the piece remaining but in the whole piece, including the head, which plays no direct part in the formation of the new posterior end (Child, '11*b*).

Rejuvenescence may also be accomplished by starvation followed by feeding, as I showed in the paper referred to, or by any other means which determines the use as a source of energy or the breaking down in any other way of portions of the structural substance of the organism or part. My experiments led me to the conclusion that senescence in its simplest terms consists in a decrease in the rate of metabolism determined by the gradual accumulation of relatively inactive structural obstacles to metabolism, which in turn are the necessary consequence of continued metabolism under constant or relatively constant conditions and in the presence of nutritive material. If this conclusion is correct then senescence is a necessary feature of life. But senescence in the lower forms does not lead inevitably to death, for any conditions which determine the use as a source of energy or the elimination of a part of these structural substances and so make possible a higher rate of metabolism bring about rejuvenescence. It is perhaps more strictly correct to say that when the formation and deposition of these inactive substances which retard metabolism is more rapid than their break-down and removal then the organism is growing old, but whenever the processes of breaking down and elimination of these accumulations are in excess of the processes which form them the organism is growing young, provided nutrition is available for the increased rate of metabolism which is thus made possible. Sexual reproduction is not the only means by which the organism returns so to speak to or toward the starting point. Every experimental reproduction resulting from the physical isolation of a piece involves necessarily a greater or less degree of rejuvenescence.

We have then in the reconstitution of a part into a new whole after experimental isolation all the essential features of true reproduction and of inheritance. The new individual formed is physiologically and morphologically younger than that from which it originated and it possesses the essential characteristics of the species.

III. Asexual Reproduction in Nature.

In a recent paper (Child, '11*a*) I have attempted to show that at least many forms of asexual reproduction in nature are essentially similar to the regulatory reconstitution of a piece experimentally isolated. As a matter of fact I believe that all forms of asexual reproduction are of this type. The chief difference between asexual reproduction in nature and the reproduction induced by experimental isolation of pieces is that the isolation in the latter case is physical and complete, while in the former it is usually at first physiological and very often only partial. The simplest types of asexual reproduction which result from autotomy, self-laceration, etc., are identical in character with the experimental reproductions or regulatory reproductions. Here the reproductive process is initiated by complete physical isolation of a part, either through accident or in consequence of violent stimulation.

In the paper referred to above I have analyzed the concept of physiological isolation and have pointed out the different ways in which it may come about. It is impossible at this time to go into these matters in detail but a brief review of some of the chief points is essential. And first the idea of the physiological dominance and subordination of parts requires attention. Some parts of organisms are relatively dominant physiologically, others relatively subordinate. In the simpler organisms, *e. g.*, the plant, *Tubularia*, *Planaria*, etc., the anterior region or the apical region, the vegetative tip in the plant, the hydranth region in *Tubularia* (Child, '09*a*, p. 19, '09*b*), the head region in *Planaria* (Child, '11*f*) is physiologically dominant over all other parts. The question as to the nature of this dominance will be considered elsewhere.

In such cases reproduction is a relatively simple matter: within certain limits any part of the organism which becomes isolated from the dominant part either physically or physiologically undergoes changes which lead to the formation of a new dominant part, which then controls and determines the reorganization of the remainder of the piece and the result is a new whole. In short, the dominant part represents physiologically the fundamental type of reaction of the specific protoplasm. Some portion, usually the anterior or apical region, of the isolated piece returns to this type of reaction and becomes first of all a new dominant part. In correlation with this subordinate parts then develop so far as material or energy is available.

In the higher organisms conditions may be more complex. The organism may consist of several or many systems, more or less closely correlated and each with its own dominant part. Moreover, as structural characteristics become more stable the capacity for regulation decreases and subordinate parts, even though isolated, may be incapable of any regulation or may give rise merely to new parts like themselves, as for example in the case of the posterior region of the earthworm, which produces a new posterior end at its anterior end when isolated.

But whatever the complications, the existence of dominant and subordinate parts is of great importance for inheritance. The dominant part can never become anything else for it represents the fundamental reaction system of the species, but from the subordinate parts a new dominant part may arise when they are isolated from their correlation with the old dominant part. In such cases, as I shall show elsewhere, the formation of the new dominant part from the subordinate part is not the restitution of a missing part but the first step in the formation of a new individual from the material of the subordinate part. The old part does not determine that a new head or apical region shall arise, but this occurs in spite of the old part. In general the weaker the old part the more readily does the new head or apical region form and vice versa. The results of my experiments soon to appear demonstrate this beyond a doubt. As regards the formation of a new posterior region from more anterior parts the case is quite different. The posterior region is subordinate to regions anterior to it, consequently the weaker the old part the less readily does the new posterior region arise from it and vice versa. It is possible experimentally to accelerate or even to determine the formation of a new anterior region from a piece by decreasing its rate of metabolism and by the same means to retard or inhibit the formation of a new posterior end.

Secondly, there undoubtedly exists, at least for certain correlative processes a distance-limit, a limit of effectiveness, beyond which the correlative influence is ineffective. Some evidence upon this point I have presented in the paper referred to. This limit of effectiveness is not absolute but varies with the rate of metabolism in the part where the correlative factor originates, with the character of the path of correlation and with other conditions. In various cases we can alter this limit experimentally. On the other hand, the sensitiveness or receptivity of a part to a correlative factor may itself vary according to conditions in and about the part serving as receptor.

From these facts it follows that the physiological isolation of parts of organisms in nature may occur in various ways: first, increase in size beyond a certain limit may isolate physiologically certain parts from the influence of a dominant part and so lead to reproduction. Second, decrease in the metabolism of a dominant part may decrease the effective distance of the correlative factor and so lead to the physiological isolation of parts without increase in size of the whole. Third, changes in the character of the path of correlation may decrease the distance to which conduction is possible and likewise bring about the physiological isolation of parts. And finally, changes in the receptiveness of subordinate parts may themselves isolate such parts physiologically.

In organisms where the parts in question are capable of regulation such physiological isolation, however brought about, will be followed by a regulatory reconstitution of the isolated part, which may lead either to the formation of a new complete individual, or a new part. The result of the physiological isolation of a part depends upon its degree of differentiation and its regulatory capacity as well as upon the degree and kind of physiological isolation. In my earlier paper examples of the various types of physiological isolation were given and various phenomena of asexual reproduction were analyzed in the light of these ideas.

In many cases the asexual reproductive element appears in a more or less highly differentiated form, *e. g.*, various forms of spores, and in some cases it gives rise to something different from that of which it originally formed a part, as for example in plants, where the spore of the sporophyte gives rise to the gametophyte. Such cases, however, do not in any way conflict with the general conclusion that asexual reproduction is the result of physiological or physical isolation of parts.

If these conclusions are correct, asexual reproduction in nature is essentially similar to the process of reproduction which occurs when we isolate physically a piece of an organism. In the natural process, however, the degree and kind of physiological isolation and the localization of the part isolated are all determined by natural conditions, either internal or environmental.

Moreover, the same considerations apply to the phenomena of segmentation and reduplication of parts for these are manifestly merely cases of partial reproduction.

In all of these cases physiological isolation is followed by a more or less complete reconstitution, according to the degree and character of the isolation. The result of the physiological isolation is then essentially the same as the result of physical isolation: the isolated part undergoes first a dedifferentiation and loses more or less completely its original characteristics as a part and then redifferentiates into a whole, or in cases of segmentation and reduplication of parts, into a new part. In the cases which are commonly regarded as reproductions the physiological isolation is usually followed sooner or later by complete physical isolation.

We have now to ask the question whether rejuvenescence occurs in asexual reproduction in nature. For certain forms, *e. g.*, *Planaria* (Child, '11*b*) and some cœlenterates, I have determined experimentally that rejuvenescence does occur in asexual reproduction. As regards the plants there can be no doubt that the same is true: the bud and the new organism arising from the spore both represent conditions nearer the starting point of the developmental cycle than the individuals of which they originally formed a part. If my conclusions stated above concerning the nature of senescence and rejuvenescence are correct there can be no doubt that in every case where a dedifferentiation and redifferentiation follow the physiological isolation of a part a greater or less degree of rejuvenescence occurs.

And finally, there is no more reason to assume the continuous existence of a hypothetical germ plasm in asexual reproduction in nature than in the case where reconstitution follows the experimental physical isolation of a part. After isolation the part may return to or approach the condition of undifferentiated totipotent protoplasm, but the assumption that undifferentiated protoplasm exists in it continuously is wholly gratuitous and superfluous.

IV. Sexual Reproduction.

We turn now to the problem of sexual reproduction. Here a number of questions require consideration. First, do the gametes arise from an undifferentiated germ plasm which is independent except for nutrition of the soma or are the gametes physiologically integral parts of the individual? Second, do the gametes before fertilization actually consist of undifferentiated germ plasm or are they differentiated cells like other parts of the organism: in other words are the gametes physiologically young or old cells? Third, why does the egg usually require the special stimulus of fertilization or some artificial stimulus in place of it for the initiation of development? Fourth, why are some eggs naturally parthenogenetic and what is the nature of artificial parthenogenesis? Fifth, how shall we interpret the alternation of generations in plants and metagenesis in animals? Sixth, what is the relation between asexual and sexual reproduction and why is sexual reproduction the only method of reproduction in the higher animals? We shall consider these questions in order.

1. *The Origin and Formation of the Gametes.*

The fact that the primitive germ cells can be distinguished very early in development in certain animals, *e. g.*, *Ascaris*, *Cyclops* and various vertebrates, is familiar to all and has commonly been regarded as strong evidence in support of the germ plasm hypothesis. In those cases in which the germ cells do not appear until the adult stage is reached it is of course easy to assume that the germ cells are there throughout development, but are simply not visibly different from other cells during the earlier stages. But is this interpretation the most satisfactory? Does it account for the facts in the simplest way? I believe that it does not.

In the first place all forms in which the germ cells are distinguishable in early cleavage or embryonic stages are forms in which the specifications of the embryonic cells become fixed at an early stage. The germ cells, like other organs, become distinguishable early in the developmental history. The case of *Ascaris*, however, stands to some extent apart from the others, for here the first cleavage separates the egg into two cells, one of which represents the germ cells plus considerable parts of the soma, while the other represents somatic parts alone and undergoes the process known as diminution. The undiminished nucleus is commonly supposed to represent the germ plasm and in this case the hypothesis of continuity seems to be confirmed by observation.

But Boveri's recent work on polyspermic and centrifuged eggs of *Ascaris* (Boveri, '10) throws new light on this case. According to Boveri it is the cytoplasm of the egg that determines which nucleus or nuclei shall undergo diminution and which shall not. All nuclei which come to lie in the animal half of the egg undergo diminution, while those which lie in the vegetative half remain undiminished. If, as most supporters of the germ plasm hypothesis believe, the nuclear substance is the real germ plasm then how shall we account for the fact that here in *Ascaris* it is the cytoplasm that determines which nuclei shall persist as germ plasm and which shall become somatic. The cytoplasm is not properly speaking a part of the germ plasm at all, if we accept the hypothesis in its original form, but rather represents in the egg a differentiated soma, yet if Boveri's conclusion is correct the cytoplasm determines the localization of the germ plasm. Apparently then in this classical case of *Ascaris*, which has been so often quoted as an irrefutable support of the germ plasm hypothesis, the visible distinction between germ cells and soma is determined by cytoplasmic differences along the egg axis. If this is actually the case, the germ plasm is certainly not an independent entity here, but is determined in the same way as many other early embryonic differentiations, *i. e.*, by its environment within the organism.

In *Cyclops* and various other forms the "Keimbahn" is characterized only by certain cytoplasmic granules which do not appear in other cells and by less rapid division. The fact that, at least in some cases these granules originate from nuclei of other cells which have been taken up by the egg, as certain authors have shown, does not alter the case. If the granules are determining factors then the germ plasm is determined by factors in the organism external to it. If they are not determining factors the existence of a "keimbahn" indicates merely that the germ plasm normally arises from a certain part of the organism. These cases then, like that of *Ascaris*, afford no real evidence of the continuous existence of undifferentiated germ plasm.

Investigations of recent years have shown that in at least many cases among the lower vertebrates the primitive germ cells appear rather early in development and often far from their final position, which they attain by migration. The evidence from observation in these cases also is merely evidence for early differentiation or specification of germ cells, not for continuity of undifferentiated germ plasm. If the germ cells are independent of the soma why should the extensive migrations occur? They behave as if they were physiologically integral parts of the organism, rather than mere parasites upon it, for certainly their migration cannot be regarded as autonomous. Perhaps it should also be pointed out that the evidence for the origin and migration of the primitive germ cells in these forms rests entirely upon the study of fixed material. So far as I am aware, the migrations have not been observed in the living embryos. Does not at least the possibility exist that cells of this character may appear as temporary differentiations or stages in various parts of the embryo, or perhaps that cells with a low rate of metabolism possess these characteristics? But without laying too much weight upon these last suggestions, we are, I think, justified in maintaining that the early appearance of primitive germ cells in vertebrate embryos, when taken as a fact of observation, constitutes evidence for early embryonic differentiation or specification, rather than support for the germ plasm hypothesis.

On the other hand, there is a very large number of forms, both plants and animals, in which the germ cells do not appear until development is completed and then in many cases only periodically. Moreover, in many of these cases they arise from tissues which are visibly differentiated and constitute functional parts of the organism. The cœlomic epithelium of the polychætes, for example, is certainly not, if appearances have any significance, an undifferentiated tissue, yet at certain seasons or under certain conditions the germ cells arise from it. It is of course easy to assume that the nuclei of these cells always contain undifferentiated germ plasm, but this is merely forsaking fact for hypothesis.

The parenchyma of the turbellaria and the cestodes is likewise not morphologically an undifferentiated tissue, yet it gives rise to germ cells at a certain stage of development and, if my observations are correct, even the more highly differentiated muscle cells in certain cestodes may take part in producing germ cells (Child, '06).

In plants buds, which later give rise to germ cells, may arise from parts which have already undergone some degree of differentiation, but which under altered conditions dedifferentiate and produce buds.

How shall we interpret all these and many other facts of the same kind? Shall we conclude that actual observation counts for nothing and that because these apparently differentiated cells produce germ cells they must have contained undifferentiated germ plasm at all times? This is nothing more nor less than an illogical procedure. We are bound to accept our observations until we have some actual evidence which conflicts with them. And not only is there no evidence to prove that cells cannot dedifferentiate and redifferentiate, but there is abundant evidence to prove that many cells can do this. The data upon this point concerning plant cells are numerous and convincing and the occurrence of dedifferentiation has been generally accepted by the botanists as a fact for many years.

In the Protozoa the soma itself or a part of it becomes the gamete. We may of course assume that undifferentiated germ plasm is continuously present in the nucleus or micronucleus, but if this is the case why is not the protozoon always a gamete? Why does sexual reproduction or conjugation in these forms occur only periodically? Evidently it does occur only when the organism is in a certain physiological condition. How are we to account for this fact if the germ plasm is continuously present and independent of the soma?

In both plants and animals the production of gametes is associated with a certain stage of the life history. Even if the primitive germ cells appear in early embryonic life they do not develop into gametes until the growth period is completed. In many plants and some animals it is possible to induce experimentally the development of gametes and the conditions which bring about the sexual stage in these cases are conditions which decrease metabolism and bring the organism into a state physiologically similar to more or less advanced old age. The extensive work of Klebs and others on plants affords the strongest evidence upon this point. All the facts point to the conclusion that sexual reproduction is a process characteristic of the physiologically old organism. If the germ plasm is continuously present and independent of the soma why should sexual reproduction always be associated with a certain stage of development? Why should it not occur earlier in one individual and later in another? To maintain that the germ plasm cannot obtain sufficient nutrition for its needs until the activity of the soma is on the decline is certainly not in accord with the facts, for even during the growth period the organism may store up reserves, *i. e.*, an excess of nutritive material may be present, but this does not induce sexual reproduction. The assumption of some sort of activation of the sexual organs is merely an assumption of the existence of physiological correlation between the parts concerned and is incompatible with the assumption of independence. On the other hand, we are well aware that, at least in many cases, the gonads when present, influence the metabolism of the soma.

The facts then point almost irresistibly to the conclusion that the formation of gametes is a function of the organism, of the soma, like the formation of leucocytes or any other organ. When the gonads arise early they develop as integral functional parts of the individual and not as parasites. When they arise late or periodically, they arise from parts which have previously been functional parts of the organism and which undergo dedifferentiation and redifferentiation to form gametes in consequence of altered correlative conditions which in turn are associated with advancing senescence.

2. *The Differentiation and Physiological Condition of the Gametes.*

It is customary to think of the gametes as consisting of or containing "undifferentiated germ plasm" all ready to develop into a new organism after fertilization. What are the actual facts concerning this point? The primitive germ cell, as a matter of fact is more or less similar in appearance to what we commonly call undifferentiated or embryonic cells, but with the beginning of the growth period the germ cell enters upon a period of differentiation. In the case of the egg this period involves an enormous increase in size and often a very great degree of cytoplasmic differentiation. In most cases also the nucleus itself becomes differentiated and chromatin disappears from it completely or almost completely. If such a cell as this were found elsewhere in the body and had no reproductive function there is not the slightest doubt that we should regard it as a very highly differentiated and specialized cell.

The spermatozoon is certainly not an undifferentiated cell according to the usual criteria. It is one of the most highly differentiated cells that we know of in the organism. Its differentiation has proceeded along different lines from that of the egg cell, but it is none the less differentiation. Even the chromatin is in a condition different from that in any other cell.

What evidence is there that either of these cells contain "undifferentiated germ plasm?" If we confine ourselves to facts they are among the most highly differentiated cells known.

Morphologically then the gametes are highly differentiated cells. What is their physiological condition? Are they physiologically old or young? As noted above, my experiments with Planaria and other forms have led me to the conclusion that senescence consists morphologically in the accumulation of relatively inactive substances or in an increasing density or impermeability or more strictly a decreasing capacity of the colloid membranes to become permeable under stimulation as a necessary result or incident of continued metabolism under relatively constant conditions and in the presence of nutritive material. Usually the inactive substances appear as more or less stable structural features, as differentiations, and their accumulation or increasing density presents increasing obstacles to metabolism. Physiologically then, senescence consists in a decrease in the rate of metabolism in consequence of the increase of structural obstacles to metabolism. The cell becomes less irritable, less capable of stimulation, and if the process goes far enough death or quiescence is the result.

This view of senescence is somewhat different from the theory recently put forward by Minot (Minot, '08). For Minot cytomorphosis, *i. e.*, the increase in cytoplasmic as compared with nuclear material is the important factor in senescence. The two views have, however, much in common, for the accumulation of structural substances usually means increase of cytoplasm, but it is possible for a cell or an organism to grow old without any such increase in bulk of the cytoplasm, merely in consequence of the increase in density and impermeability of its membranes, or in consequence of loss of water.

Moreover, I am unable to accept Minot's conclusion that no cells except the egg undergo rejuvenescence. I have pointed out above that rejuvenescence undoubtedly occurs in many somatic cells in the lower organisms and I believe the same is true, at least for certain cells, even in mammals. In gland cells for example, the loading of the cell seems to me to represent in all respects a period of senescence, ending in almost complete quiescence. With the beginning of the discharge of the accumulated material rejuvenescence begins and at the end of this period the cell is both morphologically and physiologically young, though not necessarily embryonic, that is to say, the process of rejuvenescence which it has undergone does not necessarily result in the disappearance of its characteristics as a gland cell, but it has undoubtedly become a young gland cell.

According to Minot, the egg cell at the end of its growth period is an old cell because the amount of cytoplasm in relation to the nucleus is very great. According to my criteria of senescence the egg cell is old and approaching death because the accumulation of structural material in it is so great that metabolism is reduced almost to a minimum. We know that the animal egg at the end of the growth period is almost quiescent. Warburg ('10) has shown that its oxygen requirement is very low as compared with that after fertilization. It is evident that it is capable of but little further metabolic activity. After its isolation from the body it undergoes at most only the two maturation divisions and then unless fertilized or otherwise stimulated to renewed activity it dies after a short time.

Even the maturation divisions show certain features that indicate a low rate of metabolism. In the first place they are not separated by a period of nuclear growth: apparently nuclear synthesis is impossible. And secondly, although we know little of the physiological significance of the peculiar chromatic phenomena, yet there are some indications that they too are indicative of a low rate of metabolism. Haecker, for example has induced the appearance of paired chromosomes in somatic cells by the use of ether, and various other cases of the appearace of tetrads and heterotypic mitoses in somatic cells have been recorded, in some of which the phenomena appear to be associated with conditions which decrease metabolism. According to this view then the peculiar characteristics of the maturation divisions are connected with the physiological condition of the gamete. The stimulus to these divisions is apparently in many cases the change in environment, physiological or physical, consequent upon complete isolation from the parent body. In many other cases, however, this stimulus is apparently not sufficient to bring about maturation or its completion and this does not occur until the sperm enters the egg.

Turning now to the spermatozoon, we find that its history is different from that of the egg. Here there is no such enormous increase in volume as in the egg, but maturation divisions of the same type as in the egg occur. In the later stages of the development of the spermatozoon the cytoplasm of the sperm cell is in part used up in metabolism and in part converted into stable structural substance. The nuclear substance of the spermatozoon is evidently in a very different condition from that of the egg. The sperm nucleus is separated from the external world only by thin membranes. There is no evidence that the spermatozoon, even in a nutritive fluid, can make new cytoplasm and return to the condition of an ordinary cell. Apparently it is irrevocably committed to the function of converting other forms of energy into energy of locomotion. In short, the spermatozoon is apparently also approaching death. Its life may be prolonged by certain conditions or by quiescence for a considerable period, but sooner or later it dies unless it enters the cytoplasm of an egg.

But shall we call the spermatozoon an old or a young cell? According to Minot's criterion it must be a very young cell, since it consists very largely of nuclear material. We know but little concerning the rate of metabolism in the ripe spermatozoon as compared with earlier stages, but in all probability it is low. The energy necessary for movement is undoubtedly slight and it is not at all improbable that this energy may be furnished by substances in the secretion or in the water which surrounds the spermatozoon. Synthesis is apparently impossible for the isolated spermatozoon: its activity is limited to one highly specialized type. Meyerhof ('11) has recently shown that in the sea urchin, *Strongylocentrotus lividus*, the respiration of a certain quantity of sperm at 19° C. decreases about thirty per cent. in four hours after removal from the body. This fact indicates at any rate that the metabolism of the spermatozoon decreases after its isolation from the body.

I believe that the spermatozoon represents an extreme case of that type of senescence which is associated, not with the accumulation of actual volume of substance but with increasing density or impermeability. In no other cell is the nuclear substance in so dense a form and certainly this condition is the most unfavorable conceivable for metabolism, and in no other cell is the cytoplasm so completely converted into stable structural elements.

If these conclusions are correct then the egg and the spermatozoon in their most highly differentiated forms represent the two extreme types of the process of senescence. Attention must, however, be called to the fact that some eggs and some spermatozoa are much more highly differentiated than others. In many of the smaller eggs increasing impermeability of the membranes may be as important a factor in senescence as the increase in actual volume. In some spermatozoa, on the other hand, considerable cytoplasm remains. But the important fact in all cases is that both gametes are highly differentiated cells and that their structural characteristics, whether these consist in accumulations of inactive substance or of impermeable membranes or of high density, have apparently reduced their metabolic activities to a minimum and made synthesis impossible. In short, both gametes appear to be in an advanced stage of senescence and both as isolated cells are approaching inevitable death. Certainly there is no indication that they consist of or contain undifferentiated germ plasm.

3. *The Nature and Result of Fertilization.*

In experimental reproduction resulting from the physical isolation of pieces and in asexual reproduction in nature the physical or physiological isolation of the part is usually sufficient to initiate the processes of dedifferentiation and rejuvenescence. In the case of the gametes in animals, however, differentiation of the cells has in most cases proceeded so far and the specialized structure has become so firmly fixed that physical or physiological isolation does not constitute a sufficient stimulus to initiate dedifferentiation. The maturation divisions represent perhaps an attempt of the cells to begin the process, but they are ineffective since nuclear synthesis is no longer possible and the cells continue to approach death, for the egg is unable to use its accumulated substance as a source of energy and the spermatozoon possesses no material or only a minimal amount which can serve as a source of energy.

But with the entrance of the spermatozoon into the egg conditions are at once changed. The metabolism in the resulting zygote in animals usually undergoes a rapid increase and the structural material of the egg cytoplasm, which under previously existing conditions remained inactive, now begins to enter metabolism and to serve as a source of energy. The sperm nucleus now finds itself surrounded by abundant nutritive material under such conditions that it can be used in synthesis. Moreover, as the cytoplasmic material of the egg is used up, the obstacles to metabolism become less and less and the rate of metabolism becomes higher and higher, *i. e.*, rejuvenescence proceeds at an increasingly rapid rate. In the sea urchin, as Meyerhof ('11) has recently shown, the oxidation processes are four times as great in amount in the larvæ shortly after the swimming stage is reached as in the first hour after fertilization. In those cases where the zygote forms a "resting stage," either the process of rejuvenescence goes on very slowly or else the external conditions which lead to the formation of gametes retard or inhibit the development at an early stage.

Whether the primary effect of the entrance of the sperm is a superficial cytolysis, as Loeb believes, or an increase in permeability, as R. S. Lillie and others have suggested, the result is in general the same. Materials which under the conditions existing during the developmental history of the egg cell accumulated in the cytoplasm now become available as nutrition. During its developmental stages the egg cell was in the presence of abundant nutritive material brought to it by the activity of the parent organism and its chief activity consisted in accumulation of substance until the obstacles to metabolism resulting from this accumulation or from increasing impermeability of membranes made further metabolism impossible. At the time of fertilization external nutritive material is not present or is present only in minimal quantities, consequently, and in accordance with chemical laws, the activity of the egg, as soon as increased permeability or cytolysis has made such activity possible, consists largely in the breaking up of the previously accumulated material. It is not necessary to assume that any mysterious transformation in the nature of the egg has occurred: its activity merely changes with the presence or absence of external nutritive material. The case is not very different from that of the planarian which, in the presence of sufficient food, increases in size and accumulates material and grows old, but when starved uses up the previously accumulated material, decreases in size and becomes young, at least morphologically (Child, '11*b*). The entrance into the egg in fertilization of another nucleus, the sperm nucleus, which is in a condition somewhat similar to that of extreme starvation undoubtedly accelerates the process of dedifferentiation and rejuvenescence in the egg, but it does not necessarily have any other effect so far as the initiation of development is concerned.

Fertilization then saves both the egg and the sperm nucleus from death and initiates the process of dedifferentiation and rejuvenescence by making further metabolism possible, either in consequence of increased permeability or partial cytolysis. The evidence seems to me to point toward the conclusion that the primary influence of the sperm is an increase in permeability rather than a cytolytic action, but whichever alternative is the correct one the significance of fertilization remains the same.

As dedifferentiation proceeds and the obstacles to metabolism decrease in the egg, metabolism becomes more and more rapid.

The increased metabolic activity of the egg after fertilization has been demonstrated by various investigators and we know that the rate of cleavage increases from the early stages onward, at least up to a certain stage. During this stage of metabolic acceleration the zygote is undergoing rejuvenescence, not simply because the amount of nuclear material in proportion to cytoplasm is increasing, as Minot maintains, but because the disappearance of structural obstacles to metabolism makes more rapid metabolism possible and so permits increase of nuclear material: in other words, the increase of nuclear material is an incident or a result rather than the cause of the rejuvenescence. The increase in metabolic activity following fertilization is probably not continuous but more or less rhythmical in correspondence with the periodic changes in nuclear condition. Lyon's observations (Lyon, '04) indicate the existence of such a rhythm.

The history of the egg is in many respects similar to the history of a gland cell. During the process of loading, the gland cell is growing old and metabolism decreases until finally the loaded cell can do nothing more and is almost entirely inactive. But when the external stimulus, which corresponds to the stimulus of fertilization in the egg, reaches the gland cell rejuvenescence begins. The accumulated inactive substance becomes active, but in the case of the gland cell is simply eliminated from the cell and finally what remains of the cell is young again and capable of a new metabolic cycle.

It is an interesting speculation as to whether the egg is not after all closely related to the gland cells. If a gland cell should become isolated from the organism under conditions which render continued existence possible and if it should, in consequence of the external stimulus, undergo rejuvenescence to such an extent that it lost its special characteristics as gland cell and became more or less embryonic, it would certainly develop into a new individual in much the same manner as the egg.

In the case of the developing embryo there comes sooner or later a time probably in most cases after the young animal begins to feed, when the inactive structural products of the renewed metabolism begin to make themselves felt as obstacles to metabolism. From this stage on the process of senescence once more becomes apparent in the decreasing rate of metabolism. This stage where the process of accumulation of new structural substance begins to overbalance the process of removal of the old substance is undoubtedly different in different organisms, but in all cases it occurs relatively early in development and during most of its developmental history the organism is growing old.

Incidentally it may be noted that if we accept these conclusions it is quite unnecessary to regard growth as an autokatalytic process as various authors have done. The period of acceleration in growth is simply the period during which the removal of obstacles to metabolism overbalances the development of new obstacles, in other words it is the period of rejuvenescence, and the period of retardation begins when the obstacles resulting from continued metabolism become sufficient to retard the reactions; it is the period of senescence.

In certain forms with larval stages there is a second period of rejuvenescence at the time of metamorphosis, though in many cases the larval structures or some of them are too far advanced in senescence to undergo dedifferentiation and are resorbed or cast off, the further development being taken up by cells whose metabolic activity has been previously more or less completely inhibited. I believe that in general metamorphosis is the consequence of senescence of the larval structures.

To sum up: fertilization initiates the process of rejuvenescence in the egg cell and the sperm nucleus which have previously become so highly differentiated and so old in the organism of which they formed a part that physiological or physical isolation from the organism is insufficient to initiate the process of rejuvenescence. According to this view then sexual reproduction does not differ in its essential physiological characteristics from asexual reproduction or from the regulation of artificially isolated pieces. In all these cases the differentiation of the part which forms the reproductive element or system is determined by its correlation with other parts of the parent organism and reproduction is initiated by a process of dedifferentiation and rejuvenescence. In asexual and experimental reproduction the isolation of the part is usually a sufficient stimulus to initiate the process of rejuvenescence, while in the case of the gamete a further stimulus is usually necessary.

4. *Natural and Artificial Parthenogenesis.*

In some cases the development of the egg into a new organism begins at once when it is isolated from the parent organism and without fertilization. How shall we account for these cases of natural parthenogenesis? The behavior of parthenogenetic eggs forces us, I believe, to the conclusion that they are cells which, although visibly differentiated in the same direction as the true gametes, are nevertheless not so highly differentiated nor so old physiologically as eggs requiring fertilization. They resemble asexual reproductive cells or cell masses in that the stimulus resulting from physiological isolation from the parent organism is sufficient to initiate the process of dedifferentiation and rejuvenescence.

In this connection it is of interest to note that in cases where a single individual produces both parthenogenetic eggs and eggs requiring fertilization, the parthenogenetic eggs are produced earlier in the life of the parent organism than the eggs requiring fertilization. In other words, the eggs produced at earlier stages are, like other parts of the organism, not so highly differentiated nor so old physiologically as those produced in later stages. This fact of the relation of parthenogenetic and non-parthenogenetic eggs to the life cycle of the parent organism seems to me to constitute one of the strongest arguments in support of the view that the gametes are physiologically integral parts of the soma and that they differentiate and grow old in the same manner as other parts; moreover, I know of no other way of accounting for the fact.

The eggs of some species, if left for a time without fertilization, often show the beginnings of more or less normal development, but sooner or later die. Such eggs are intermediate between the true parthenogenetic and non-parthenogenetic eggs. They react to the stimulus of isolation by a slightly increased metabolism but the reaction is either insufficient to establish the processes so that they continue, or else the stimulus of isolation is not entirely adequate to initiate processes which are in all respects of normal character.

In a few cases it has also been observed that the last eggs of the breeding season show some indications of parthenogenetic development. These are doubtless eggs which have remained for a relatively long time in the ducts of the parent organism. Under these conditions the egg is cut off from its supply of nutrition, but is still under conditions more favorable to continued existence than those of the external world. As long as the egg continues to live a minimal amount of metabolism is undoubtedly going on. In the absence of external nutrition this metabolism itself must bring about a slight degree of dedifferentiation and when these eggs finally emerge from the ducts they are probably slightly younger than those extruded earlier and therefore react somewhat more strongly to the changed conditions by showing some indications of parthenogenetic development.

The work of recent years on artificial parthenogenesis has demonstrated that other means of stimulation of the egg to development may be substituted for the spermatozoon and it is, I think, sufficiently demonstrated that the essential feature of these artificial methods of stimulation lies in the fact that they make possible a continuation and increase in the metabolism of the egg. Whether they accomplish the result through superficial cytolysis or through an increase in the permeability of the membrane is not of prime importance for the present purpose: it is quite possible that some act in one way, some in the other. Certainly it does appear to be true that many of the parthenogenetic agents do increase the permeability of the membranes and if R. S. Lillie's view (Lillie, '09*a*, '09*b*, '11) that the stimulation of a cell consists essentially in increase of permeability to CO_2 is correct, it is not improbable that the effect of at least many of the parthenogenetic agents on the egg is of this nature and therefore not in any sense specific. According to the view of reproduction developed in this paper, there is absolutely no reason to suppose that it is specific. Moreover, I cannot agree with Loeb ('09) that it is any more a formative stimulus than any other. It is primarily a stimulus to metabolism and the formative changes are an incident or result of continued metabolism.

But in any case the methods and agents of artificial parthenogenesis merely serve to initiate the processes of dedifferentiation and rejuvenescence in the egg by bringing about increased metabolism. Isolated pieces of *Planaria* which are "too small," when kept at a certain temperature, to regulate into new wholes can be made to undergo such regulation by raising the temperature a few degrees and by various other methods. I believe that such cases are not fundamentally different from cases of artificial parthenogenesis.

5. *Alternation of Generations in Plants and Metagenesis in Animals.*

In many of the lower plants in which sexual reproduction is known maturation is not immediately followed by fertilization, but the mature cells are spores which pass through a developmental cycle and this results in the formation of the gametophyte generation: this finally produces the gametes, which after fertilization give rise to the sporophyte. In the higher plants the gametophyte generation is much reduced and never becomes an independent, free living organism. The sporophyte produces two kinds of spores, the microspore or pollen grain and the macrospore or embryo sac. These structures in their further development represent all that remains of the gametophyte and they give rise to the gametes. In many plants then there are two reproductive cycles instead of one, although in the higher plants one of these cycles is much reduced.

The production of spores by the sporophyte is apparently associated with the senescence of this generation or of parts of it and as Klebs and others have shown can often be induced, at least after the earlier stages of the vegetative period, by conditions which decrease metabolism. But the spore resembles the parthenogenetic egg in that it reacts to the stimulus of isolation either with or without a period of quiescence by initiating a developmental cycle. It differs from the parthenogenetic egg, however, in that this developmental cycle is different from that in the course of which it arose. The spore then is not so highly differentiated or so old that it is unable to react to the stimulus of isolation by a regulatory process, but this process gives rise to an organism of different character from the preceding generation. In short, a second cycle of dedifferentiation and rejuvenescence occurs between maturation and the formation of the gametes. The dedifferentiation in this case, however, does not carry the organism back to the original starting point. The spore, which arises from a part of the sporophyte has come to possess different capacities from the zygote and these become evident in the gametophyte into which it develops. There is certainly no reason for believing that the spore consists of or contains undifferentiated germ plasm. On the basis of such an assumption its development into a gametophyte becomes inexplicable. It seems to me that we can regard the spore only as a differentiated part of the sporophyte which in the course of regulation following its isolation from the sporophyte body does not entirely lose the physiological characteristics which it has acquired and therefore produces something different from that of which it formed a part. Moreover, in cases where apospory occurs parts of the sporophyte give rise to gametophytes without the formation of spores and in some cases regeneration in the sporophyte gives rise to gametophyte-like structures. Evidently then other parts of the sporophyte than those which normally form spores are specified in the direction of gametophytic development, but only in certain cases or under certain conditions do such parts become physiologically isolated.

In many forms also the gametophyte may reproduce asexually, giving rise to new gametophytes by various forms of budding. Here, as in the sporophyte, with every such reproduction a greater or less degree of dedifferentiation and redifferentiation undoubtedly occurs.

The existence of this type of life cycle shows very clearly that there is no immediate or necessary connection between maturation and fertilization. In these plants the maturation divisions are followed by the development of a new individual in which the cells all possess the reduced number of chromosomes. Since we know practically nothing concerning the physiological significance of the maturation process, the reason for its occurrence in connection with spore-formation is not apparent. The process seems, however, to be associated with a low rate of reaction and with conditions which prevent nuclear synthesis.

As the gametophyte becomes old it in turn gives rise to gametes, which, as in the animal are, so far as appearance goes, highly differentiated cells and if their behavior is any criterion are also physiologically old. And here in order that anything further may occur the stimulus of fertilization is necessary.

This type of life cycle is probably connected with the great development and specialization of vegetative organs and vegetative reproduction in the plant. The sporophyte becomes so large and so highly differentiated that parts of it are physiologically or physically isolated before the stage of gamete formation is reached. These parts retain some degree of specialization during their regulation after isolation and so produce the gametophyte, which is usually much simpler morphologically than the sporophyte, but is physiologically specialized in the direction of gamete formation.

The occurrence of apogamy in the gametophytes of some species merely shows that less highly differentiated cells are capable of producing asexually the same result which in the more highly differentiated gametes occurs only after fertilization.

Alternation of generations, so-called, or more properly metagenesis, in animals is different in certain respects from alternation of generations in plants. In animals the asexual cycle always occurs before maturation and maturation and fertilization are never separated by a developmental cycle. Moreover, in animals the asexual bud which gives rise to the so-called sexual generation usually undergoes its earlier development as a specialized part of the asexual colony and becomes free if at all only when its development is advanced. Its formation is, however, undoubtedly connected with advancing differentiation and senescence in the asexual colony or in that part of it from which the sexual generation arises: in the hydroids, for example, the medusa bud very commonly arises from the most highly differentiated part of the asexual form, viz., the hydranth. It is probable, moreover, that it is initiated by the physiological isolation of a part as in other similar types of reproduction. In this respect the phenomena of metagenesis are essentially similar to those of alternation of generations in plants. In both cases there is a second reproductive and developmental cycle interpolated between the first and the formation of new gametes: in the plants this cycle occurs after maturation, in the animals before.

6. *Asexual and Sexual Reproduction in Relation to the Life Cycle.*

In general, where the same individual reproduces both asexually and sexually, the asexual method of reproduction is characteristic of the earlier, the sexual method of the later stages of the life cycle. In other words, when the organism is still relatively young, parts of the body which become physiologically isolated in consequence of increase in size or decreasing metabolism in dominant parts or other conditions (see p. 8) are capable of reacting to the stimulus of isolation by dedifferentiation and rejuvenescence, which is followed by a new developmental cycle.

But as the organism grows older its parts become more highly differentiated and undergo regulation less readily or not at all when isolated. At this stage of the life cycle the only form of reproduction which is still possible is reproduction by means of gametes. These parts of the organism are more highly differentiated than the asexual reproductive parts of earlier stages and, except in cases of parthenogenesis, require fertilization. In short, asexual reproduction is characteristic of the younger, less highly differentiated organism and sexual reproduction of the older, more highly differentiated. These facts constitute further strong evidence in support of the view that the gametes are really highly differentiated parts of the organism instead of undifferentiated germ plasm.

One of the most striking examples of the relation of asexual and sexual reproduction to the life cycle is found in certain medusæ belonging to the family Margelidæ. Reproduction in these forms has been described by Chun ('95) and later by Braem ('08). These medusæ reproduce asexually during the younger stages and sexually when older. Reproduction takes place by means of bud-like outgrowths which arise in a more or less definite sequence and arrangement on the manubrium, their formation beginning near the base and proceeding toward the tip. All the buds formed in the earlier stages of the life cycle are asexual and give rise to new medusæ. As the medusa grows older, however, the outgrowths continue to arise on the manubrium in the same order as before and in their earlier stages resemble the asexual buds of the younger animal, but they give rise to gonads instead of producing new medusæ asexually.

In this case then the same region of the body is concerned in both asexual and sexual reproduction and the early stages of asexual buds and of gonads are similar. The reproductive cells of the younger, less highly differentiated animal are themselves less highly differentiated and so develop asexually into new medusæ, while the reproductive cells which arise when the animal is older and more highly differentiated develop into the highly differentiated gametes, which require fertilization.

In the higher organisms, where the structural features are more stable and the capacity for dedifferentiation and rejuvenescence is limited asexual reproduction disappears and sexual reproduction remains as the only method of reproduction. In many of these forms, however, the absence of asexual reproduction in the earlier stages is due, not to the absence of capacity for reaction to isolation, *i. e.*, for regulation, but to the fact that under natural conditions parts do not become physiologically isolated. Experiment has shown, for example, that in the eggs and embryos of various vertebrates isolation of parts is followed by more or less complete regulation and the occasional occurrence of identical twins in man, as well as the occurrence of polyembryony in the armadillo as a natural phenomenon (Newman and Patterson, '09, '10), indicate that even in the mammals the earlier stages still retain a high capacity for asexual reproduction. It is not impossible that we may be able at some time with proper technique to induce asexual reproduction, even in parts of the older vertebrate organism.

In plants, likewise, sexual reproduction is characteristic of the old organism with a relatively low rate of metabolism and it can often be induced by bringing the plant under conditions which decrease metabolism. The fact that in the lower plants the gametophyte is usually simpler morphologically than the sporophyte does not, I believe, constitute a real exception to the general law. The sporophyte is a form in which most of the cells become at an early stage so highly differentiated that they are entirely excluded under ordinary conditions from the function of reproduction. Only those parts which remain in a relatively primitive condition or which differentiate relatively slowly play a part in reproduction. These parts are, so far as we can judge, less highly differentiated than the tissues of the gametophyte from which the gametes arise. The plants therefore are no exception to the general law that asexual reproduction occurs in the less highly differentiated, younger organism or part and sexual reproduction in the older, more highly differentiated.

It has been pointed out above that the gametes are both morphologically and physiologically among the most highly differentiated cells that we know. This is exactly what might be expected if they arise as parts of old and highly differentiated organisms. Moreover, on any other basis it is very difficult to account for the differentiation of the gametes, except teleologically. A cell consisting of "undifferentiated germ plasm" is certainly capable of forming a new organism at once, provided nutritive material is accessible. Why does not the primitive germ cell use the nutritive material which comes to it for such development rather than for a series of complex differentiations which disappear as soon as the development of the new organism begins? To assert that these differentiations are connected with the necessity of fertilization is to put the cart before the horse. We know that many plants can be bred asexually for an indefinite number of generations and some have even lost the capacity for sexual reproduction or never possessed it. Moreover, recent experimental work has demonstrated that in some infusoria the supposed necessity for periodic conjugation is a myth and that under proper conditions these animals can be bred asexually for thousands of generations and perhaps indefinitely. In experiments of my own with planarians I have already bred the animals asexually for twelve generations and during this time there has been no loss of vigor and functional sexual organs have not developed at any time. It seems much more nearly in accord with the facts to conclude that fertilization is simply a necessary consequence of the differentiation of the gametes as parts of the organism, rather than to maintain that the differentiation of the gametes is a preparation for fertilization. Besides this, it is difficult if not impossible to exclude the principle of finality from the latter view. And lastly, the sexual differentiation of the gametes which is characteristic of the higher organisms is not necessary for fertilization since in many cases where fertilization occurs it does not exist. What possible reason can there be then for this differentiation, except the reason which exists for the differentiation of any part of the soma? On the other hand, is continued metabolism without some sort of differentiation possible?

So long as we do not permit our view of the facts to be obscured or distorted by hypothesis and theory, we cannot, I believe, escape the conclusion that the gametes undergo differentiation because they form as integral physiological parts of differentiating organisms and that they are more highly differentiated than asexual reproductive cells or cell masses because they arise from older or more highly differentiated organisms than these.

Sexual reproduction is then merely the final term in the reproductive series: it is the most highly specialized type of reproduction. The gamete itself is so highly differentiated and so old that it is incapable of reproduction except with the aid of an external factor.

V. Heredity and Inheritance.

If the above conclusions are correct it follows that our theories of heredity and inheritance, instead of being based solely or primarily on the phenomena of sexual reproduction, must find their basis for analysis and interpretation of these phenomena in the simpler forms of asexual and experimental reproduction. Sexual reproduction is in many respects the most unfavorable form of reproduction for investigation and analysis of the process of inheritance, for here we find the greatest number of complicating factors, viz., the high differentiation of the gametes, the presence of large masses of non-living material in the egg and the union of different gametes. We can, it is true, subject different gametes to different conditions and observe the result of their union and this method has already given results of great interest and value, but he must be an enthusiast indeed who would maintain that the complex formulæ presented by various recent investigators afford us any real insight into the actual processes of inheritance. Their terms are symbols for something, but for what we do not know and breeding experiments cannot tell us. And to say this is not to detract in any way from the interest and value of recent work on breeding and hybridization, but is merely to state its limitations. The sexual breeding of higher animals does not and cannot at present, if ever, go much beyond the determination of empirical symbolic formulæ. We can say that the combination of x and y gives z, but all are unknown quantities and breeding experiments of this character cannot make them anything else. They do not solve the problem but merely state it in symbolic terms.

To understand, or even to formulate the processes of inheritance we must first of all know the processes in the individual and we must investigate the process of reproduction in its simplest forms. When we have done this we shall be better able to attack the problems involved in the more complex processes of sexual reproduction.

We have, I believe, in the experimental reproductions, *i. e.*, in the regulation of isolated pieces, a field of the greatest importance for the investigation of inheritance. Here we can vary the size of the reproductive element, the region of the body from which it comes, the conditions under which it shall regulate and the conditions under which the parent organism lives before the piece is isolated. And I have already shown in part (Child, '11*c*, '11*d*) and shall show further in later papers that all these factors are of importance in inheritance. Moreover the organism is primarily a dynamic system, a complex of processes, it is comparable rather to a river with its current of energy and its morphological limiting conditions (Child, '11*e*) than to a machine in the ordinary sense. Sooner or later we must interpret the organism in terms of dynamics rather than in terms of morphological entities. Many years ago Huxley said of the cells: "They are no more the producers of the vital phenomena than the shells scattered along the sea-beach are the instruments by which the gravitative force of the moon acts upon the ocean. Like these, the cells mark only where the vital tides have been, and how they have acted." These words, written more than a generation ago, have lost none of their value and may well serve to-day as a guide to biological investigation and a warning against certain types of biological theory.

Let us take the case of the chromosome, for example, which plays so important a part in recent biological hypothesis. What is the chromosome? If it is what many authors seem to believe, it is an autonomous being endowed with something more than human intelligence. But if we are not willing to believe this, then we must regard the chromosome as an incident or result of dynamic processes in the organism, like other morphological entities. If this is the correct view, then it is nothing ultimate or fundamental. We must analyze it into terms of the processes which have made it and in this analysis we shall sooner or later find nothing more nor less than the whole complex of processes which constitute the organism. The organism makes the chromosomes, not the chromosomes the organism.

Montgomery ('06, p. 56) has said that we understand heredity so far as we know the behavior of chromosomes. To my mind the exact opposite of this statement is much more nearly true. The reappearance of chromosomes in successive generations of cells is itself as truly a special problem in inheritance as the reappearance of any other characteristic morphological feature of an organism, *e. g.*, the fingers of the hand, the hand itself, etc. Moreover, to state the problems of inheritance in terms of chromosomes is nothing more than a statement and not a solution of the problem, and besides this it cannot be a complete nor a correct statement.

The experimental data of recent years on heredity are very commonly regarded as supporting and confirming the germ plasm hypothesis. And particularly the at least doubtful character of much of the evidence bearing upon the inheritance of acquired somatic characters is considered as a strong indication of the independence of the germ plasm and the soma. It seems to me, however, that the real problem is obscured by our ignorance or misconception of the nature of inheritance. A very large proportion, if not most of the individually acquired somatic characters, are due to changes in metabolism which are primarily quantitative, not qualitative in character. Such quantitative changes in the dynamic processes in the organism are dependent upon actually existing internal or external environmental conditions and cannot be expected to persist indefinitely after the conditions which produced them are no longer present. As a matter of fact, if a quantitative change in metabolism is sufficient to alter the structure to any marked degree, the effect of the change may persist in the part or parts concerned for a time or perhaps indefinitely after the conditions have ceased to act. But we cannot expect always to find characters of this sort permanently inherited, even if the germ cells are most intimately correlated physiologically with the soma, for as soon as the gametes are isolated and undergo dedifferentiation their rate of metabolism becomes very largely independent of the conditions to which they were subjected as parts of the organism. In some cases the effects of such quantitative changes may reappear to some extent for a generation or two, but they soon fade out. The non-inheritance of such characters does not then afford any evidence either for or against the independence of germ plasm and soma. In order to obtain direct evidence upon this point we must first know something of the nature of the processes which give rise to a certain character, and second, something of their correlative effect upon other parts of the body. Then we shall be able to determine what their inheritance or non-inheritance means.

It is not, I believe, too much to say that at present we have no positive evidence from the data now at hand concerning inheritance that the germ cells are independent of the soma. The established facts are simply that many individually acquired somatic characters are not inherited. We do not know, however, whether it is possible for them to be inherited even if the germ cells are integral physiological parts of the organism.

The inheritance of a character, whether it results from direct influence of factors of the external world upon the reproductive cell or cell mass or from the influence of changes in the soma, cannot depend merely upon the production of a change of any kind in the processes in the reproductive element, for many such changes disappear at once or very soon when conditions are altered. It must depend rather upon the establishment of a new dynamic equilibrium in the system and an equilibrium which is relatively stable, so that every change in conditions does not destroy it. This is doubtless the reason why so many attempts to produce mutations or new genotypes have been unsuccessful. As our knowledge of the dynamic processes in organisms increases we shall be able to effect the establishment of such equilibria more readily.

Moreover, the apparent independence of the germ cells is readily accounted for in another way, viz., by the fact that they undergo dedifferentiation and rejuvenescence after isolation and fertilization. In this process only the most stable characteristics of the specific protoplasm acquired during their history as a part of the organism remain and all else is eliminated. Two different parts of the planarian body, for example, with different degrees and kinds of differentiation, both produce, when isolated under the usual conditions, individuals with the same general specific characters, though some minor differences connected with the different origin of the pieces may persist. There are, however, certain limits to the process of regulatory reproduction in *Planaria* and other forms and an investigation of these limits and the conditions which determine them promises results of much interest for the problem of inheritance. On the other hand, it is possible to demonstrate experimentally that changes in the hereditary capacity of parts of the planarian body can be induced by changing their position with respect to other parts. If, for example, we cut off the anterior half of the planarian body and allow a head to form at the anterior end of the posterior half, we find that the capacity of the region just posterior to the new head for developing a head when isolated is very greatly increased by its changed position in the body.

The further elaborations of the germ plasm hypothesis, the hypotheses of determinants and of unit characters as represented by discrete independent elements, are wholly unnecessary. Far from assisting us in analyzing and interpreting the phenomena of inheritance, they only complicate the problem, for if they exist they are the most remarkable entities in the world. Their assumed existence makes real progress in the solution of the problems involved almost impossible, for we can juggle with them as the facts seem to demand and there is none to say us nay, since they are beyond the limits of scientific investigation. The apparent independent variation of characters, the Mendelian phenomena, the association or coupling of characters, sex-limited inheritance and in fact all the known phenomena of inheritance can be far more readily accounted for on the basis of different dynamic equilibria. If the organism is a dynamic system, changes in its constitution or in the conditions of the environment may alter its equilibrium and such changes may become evident, now in this character now in that or in a group of characters, according to the nature of the organism and the conditions concerned. There is absolutely no reason for supposing that a localized morphological character must be represented by a localized unit or entity of any sort. It may be and undoubtedly is simply a local manifestation of a condition pertaining to the organism as a whole. How can we doubt, for instance, that the color characteristics which have played so important a part in many Mendelian experiments are dependent upon the metabolism of the organism as a whole, rather than upon independent units existing in a hypothetical germ plasm. In the course of my own work on *Planaria* I have been able to demonstrate that the number, size and localization of such definite and sharply localized characters as the eyes are dependent upon, and vary with quantitative metabolic conditions existing in the whole organism or piece.

The germ plasm hypothesis gives us no help at any point. The facts can be more readily interpreted without it than with its aid: in fact it does not serve as a basis for analysis but merely affords us a means of paraphrasing the facts of observation into terms of absolutely unknown quantities. Why should biology continue to burden itself with this mass of speculation which affords no basis for real progress?

But if heredity is not the genetic history of the germ plasm or its determinants or unit characters, how then shall we define it? In the first place, we must admit, I believe, that wherever reproduction of any kind, whether of parts or of wholes, occurs, there we have also to do with heredity. And secondly, the facts seem to me to show that we are concerned in heredity rather with capacities, with potentialities, than with continuously existent entities. And finally, there is much evidence which indicates that these capacities and potentialities exist simply in the sum total of the dynamic processes which are characteristic of the organism and in the colloid field in which these processes occur. It seems to me no more necessary to postulate a specific hereditary entity for a specific morphological character of an organism than it is to postulate such an entity for a sand bar or an island developing in a river.

In a recent paper (Child, '11*c*) I suggested a definition of heredity which I repeat here since it seems to cover the ground and does not involve unwarranted assumptions. "*Heredity is the sum total of the inherent capacities or "potences" with which a reproductive element of any kind, natural or artificial, sexual or asexual, giving rise to a whole or a part, enters upon the developmental process.*" In short the heredity of any reproductive element is simply the record in its capacities of its past history. Such a statement does not, however, imply that every factor in its past has left a permanent record. If we admit that the sex cells, like the asexual reproductive elements and the reproductive elements resulting from the artificial isolation of pieces by operation, are at some stage of their development integral physiological parts of the organism then we may define heredity as *the capacity of a physiologically or physically isolated part for regulation.*

In the realization of these capacities and potentialities environmental factors, internal and external, play an important part. A "latent" character is simply non-existent as an entity, *i. e.*, it exists only in the potentialities of the system. Latent characters are no more real things than are latent sand bars and islands in a river. The organism possesses almost infinite potentialities, but what conceivable reason is there for regarding them as different from potentialities elsewhere in nature.

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PRELIMINARY NOTE ON GAMETOGENESIS IN PHILOSAMIA CYNTHIA.

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Since the history of the chromosome groups has been so completely worked out in the spermatogenesis of insects, and the fact well established of an almost universal dimorphism in the spermatozoa, it has become increasingly important to study the conditions of maturation in the eggs of insects. It has been assumed that in insecta the eggs are all alike in the number and behavior of the chromosomes, and the recent observations of Morrill ('10) and others have shown this to be the case.

In studying the history of the male germ cells in the moth *Philosamia cynthia*, the writer found ('07) that the diploid chromosome groups are reduced to exactly similar haploid groups in the spermatocyte divisions. The same facts were determined by Stevens ('05) and Cook ('10) for various other Saturnidæ.

Since no dimorphism was observed in the chromosome groups of the male, the possibility suggested itself that dimorphism might be found in the eggs. Large numbers of *cynthia* eggs were examined to determine the behavior of the chromosomes in maturation, with especial reference to the question of dimorphism. The results may be briefly stated as follows:

The number of chromosomes in the somatic cells is 26, which is also the spermatogonial number. The reduced number of chromosomes is found in the eggs just before they are laid. At this stage of late prophase, 13 chromosomes lie enclosed within the nuclear membrane, near the surface of the egg. The chromosomes are smooth, elliptical or dumb-bell-shaped bodies, well separated from one another. They show only slight differences in size and form. When arranged on the first polar spindle in anaphase, each of the dyad chromosomes separates into equal parts, which move to opposite poles of the spindle. In several hundreds of eggs examined, all the chromosome groups appeared similar. The chromosomes remaining in the egg are again halved in the second division. Thus the two polar bodies receive groups exactly like that of the egg.

At the time of fusion of the germ nuclei, just before the nuclear walls break down, 13 chromosomes may be counted in the female pronucleus, 13 in the male pronucleus, this being the number found in the spermatocytes. By fusion of the two groups, the somatic number is obtained, which is found in the ensuing embryonic divisions.

Thus on the basis of observable differences in chromosomes, there is no indication of a nuclear dimorphism in the eggs, nor of the presence of idiochromosomes.

In the papers on spermatogenesis of Lepidoptera, above referred to, the writers describe a dumb-bell-shaped body, which is distinguished from the other chromosomes by its condensed form during growth. Later it becomes indistinguishable from the other chromosomes, and divides normally. This has been interpreted as an idiochromosome in which the *X* and *Y* elements are equal. Thus the Lepidoptera, as has been previously suggested, appear to belong in the same class with *Nezara*, which possesses an equal pair of idiochromosomes in the male. If the spermatozoa are to be considered dimorphic, it is necessary to assume a qualitative difference between *X* and *Y*. However the facts may be interpreted, there is no essential disagreement between gametogenesis in *cynthia* and in other insects in which no nuclear differences are observable.

March, 1912.

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SENSORY EPITHELIUM OF PHARYNX AND CILIATED PITS OF MICROSTOMA CAUDATUM.

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University of Virginia.

For more than four years the senior writer has been able to collect *Microstoma caudatum* Ldy. from a little ice pond at the eastern border of Charlottesville, Va. These animals during the late autumn and on into early winter are frequently found sexually mature; all that we have found in such condition proved to be females. A mature female is indicated in our first figure, plate I. No males have been recognized by either of us. Many specimens have been sectioned and their sections studied in series. These studies have shown that the sexually mature individuals were not hermaphroditic.

Specimens have been fixed in hot aceto-sublimate, Bouin's fluid, and chrom-aceto-formalin mixture. In all cases the last medium has given us the best results. We think it highly important that the specimens be handled individually in fixing. Our studies of the histology of the ciliated pits have been based upon material fixed in chrom-aceto-formalin for twenty-five minutes. Specimens were then rinsed in two or three changes of tap water and carried through to paraffin. Sections were made from three micra to ten micra thick. Iron hæmatoxylin with Bordeau red as a counter stain was employed.

Microstoma caudatum Ldy. has a spindle-shaped body measuring from 750 micra to 1.5 millimeters in length. Its anterior end is more rounded than the pointed, posterior end. The entire surface is highly ciliated. The mouth, leading into a conspicuous, ciliated pharynx or œsophagus, lies on the ventral side about one sixth of the length of the body posterior to the anterior end. No eyes or "eyes-spots" have been observed by us. The ciliated pits lie dorsal to the mid-lateral surface of the body a little anterior to the mouth. These organs together with the pharynx can be closed or distended. When the animal is testing the water the ciliated pits and the pharynx are opened and closed in such a manner as to greatly alter the contour of the anterior end. These then constitute invaginated regions of the body-surface which test the character of the water passing over the body and into the pharynx. This being the case it is of interest to see the relation of the central nervous system to the epithelia of the pharynx and of the ciliated pits.

The central nervous system of *Microstoma caudatum* Ldy. consists of two anterior ganglia connected by a very short, wide, transverse commissure. Extending from each ganglion is a dorsal, lateral nerve and a ventral, lateral nerve. The ventral, lateral nerves are connected by a commissure just posterior to the pharynx (text-figures 1 and 2, *N*). In the posterior floor of the pharynx there is a transverse region of its ciliated epithelium, which, so far as we can make out, is free from any ducts of

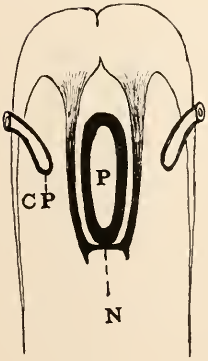

FIG. 1.

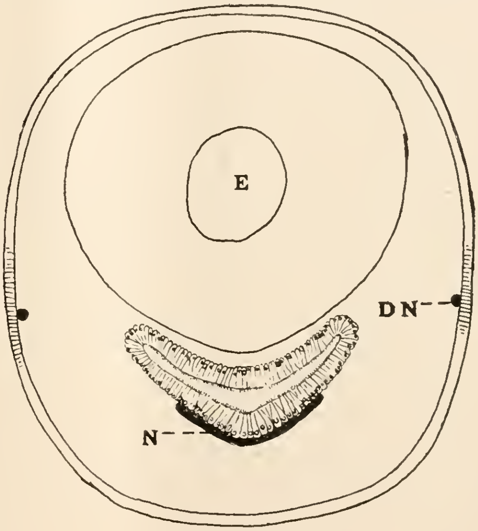

FIG. 2.

FIG. 1. Diagram of central nervous system showing its relation to ciliated pits and pharynx. *CP*, ciliated pit lying over dorsal, lateral nerve; *P*, pharynx; *N*, posterior commissure connecting the two ventral, lateral nerves.

FIG. 2. Diagram of a transverse section of *Microstoma* to show the position of the gustatory epithelium and the posterior nerve commissure. *N*, posterior nerve commissure lying in contact with the gustatory epithelium of pharynx; *DN*, dorsal, lateral nerve; *E*, lumen of enteron.

the unicellular glands of the pharynx, but which has its cells resting directly upon a transverse commissure connecting the ventral, lateral nerves (text-figures 1 and 2, *N* and Fig. 3, *N*). Martin ('08, Fig. 10) has shown how the pharynx of *Microstoma caudatum* Ldy. is everted when about to ingest a *Hydra*. In a similar way we have seen *Microstoma caudatum* Ldy. evert its pharynx in testing the edible or non-edible quality of plants and debris about which it was swimming. Judging from the intimate morphological relation between this band of pharyngeal cells and the nerve commissure and the manner in which the pharynx is operated, we believe we have here a *primitive gustatory organ.*

The ventral nerves in passing along the right and left sides of the pharynx might have made similar contacts with the pharyngeal epithelium and thus the gustatory organs would have been in this case bilaterally arranged, but in such case they would not be directed at right angles to what is the usual source of stimulus due to the animal moving anteriorly and to the food falling upon the floor of the pharynx. Thus we find this very primitive gustatory organ, in a free-moving bilaterally symmetrical animal, to be a median structure.

The ciliated pit when closed is a club-shaped sac which rides the dorsal, lateral nerves (text-figure 1, *CP*). Its lumen is lined with cilia much stronger than the cilia of the general surface. These are especially heavy near the mouth of the pit. The ciliated columnar cells of the ventral side near the mouth lie in intimate contact with the dorsal, lateral nerve (Fig. 4, *DN*). Hence we consider them to be the peculiar sensory cells of the ciliated pit, though we can see no other striking difference between them and the other ciliated cells of the outer half of the pit. Men have always looked upon these structures as being organs of special sense. It is important to state in this connection that here we have the same habit as the pharynx displays of repeatedly exposing the sensory cells to contact with stimuli by opening and closing the pit and the same kind of nerve supply as we have in the median gustatory epithelium of the pharynx. Their reason for being bilaterally placed is suggested in the latter part of this paper.

The epithelium of the ciliated pit, just as that of the pharynx, represents a modified region of the general body-epithelium. This differentiation can be studied in specimens undergoing binary fission. Fig. 2 represents a ciliated pit in a newly-forming individual. In this it is shown that as the flat, pavement epithelial cells of the general surface pass into the wall of the embryonic pit they become taller until at the fundus or blind end of the pit there are very tall, columnar cells. As differentiation proceeds, however, we get a point of great systematic interest that has not been made so far as we have been able to determine. The cells at the fundus of the growing pit continue to grow and differentiate themselves as unicellular glands. Thus in the fully developed pit we have its fundus composed of large, glandular cells which lie deeply embedded in the mesoderm. These cells have pear-shaped bodies measuring 10 micra to 12 micra in diameter. Each cell has a glandular duct leading into the lumen of the pit (Fig. 4, *G′*). It is not therefore as von Graff ('09, Seit 64) says of rhabdocœles that "Die Grübchenflecken sind Hautstellen, die keine Rabdoide und Drüsenausführungsgänge besitzen"; nor is the histology of Wilhelmi's "Auricularsinnesorgane" described as being so differentiated. In the mature ciliated pit of *Microstoma caudatum* Ldy. we have a differentiation of its cells into a sensory and a glandular region (Fig. 4). The apparent cuticula shown in this figure is but the cut ends of cells radiating from the plane of the section, and it cannot be considered analogous to the "homogeneous mass" which Ott ('92) found covering the ciliated ends of the cells in the ciliated pit of *Stenostoma leucops* O. Schm.

As stated above this has considerable systematic importance. Zoölogists look upon the affinity between Turbellaria and Nemertini as being very strong. The "cerebral organs" of Nemertini have been considered the homologues of the ciliated pits of Turbellaria. These "cerebral organs" in the highest Nemertini show no resemblance to the ciliated pits of any turbellarian but the "cerebral organ" "in its simplest form, in the Protonemertini, is a mere groove in the epidermis not extending deeper than the basement membrane; it is lined by ciliated cells, and at the bottom are large nerves from the brain" (Benham, '01, p. 185). So it appeared that the simplest "cerebral organs" differed from ciliated pits only in that the former were differentiated into a glandular region and a sensory region. In *Microstoma caudatum* Ldy. we find a similar differentiation into glandular and sensory regions. Thus we see the affinity between Turbellaria and Nemertini strengthened by the structure of the ciliated pits of *Microstoma caudatum* Ldy.

So much for the morphological part of the present paper. The second part has to do with experiments performed upon *Microstoma.*

We have collected *Microstomas* by placing into an aquarium sticks, leaves, and other submerged objects that have been taken from the marginal bottom of the pond. It is only after the filled aquarium, containing the debris from the pond, has stood twelve to twenty hours that the *Microstomas* appear at the surface of the water. In making collections for histological material it was noticed that the animals were quite alert and active when they first appeared at the surface. Under laboratory conditions bacteria accumulate rapidly in the aquaria so that within forty-eight hours a thin film appears at the surface. After this film has appeared the *Microstomas* seem to be less alert, and when this film has become dense the *Microstomas* must be actually touched in order to be caused to move from the place in which they were lying. This observation was made only after a point brought out by some of the experiments, to be described below, had indicated it.

If specimens are collected as soon as they appear at the surface and studied under supported cover-glasses by means of the compound microscope they are seen to make numerous exploratory movements as they swim about by thrusting their anterior ends to and fro, thus testing the water by means of their ciliated pits and pharynx. These actions then may be taken to indicate the nearly normal response of *Microstoma* to nearly normal conditions.

Next some specimens were placed in a .05 per cent. salt solution to see what their response would be to less normal conditions. It was found that the exploratory movements were intensified and the ciliated pits widely distended with each anterior thrust of the body. Thus it was seen that a change in conduct could be induced by artificial media.

In casting about for reagents with which to further experiment we decided to try dilute acetic acid because these specimens were found among decaying vegetable matter in which we inferred acetic acid might be present. Specimens were placed in eosin water.[1] One of these in eosin water was placed under a supported rectangular cover-glass and by means of a capillary pipette, mechanically controlled, a drop of 1/25 per cent. acetic acid added. The specimen swam about until it came in contact with the colorless margin of the drop of acetic acid solution when it reacted by turning directly away from the acid as indicated by text-figure 3. This experiment was repeated eighteen times with practically the same results. When the animals came near the zone of acetic acid their anterior ends would be expanded to such a great extent as to make them distinctly three-lobed. When the acid was reached the anterior end would be greatly distorted while the posterior end would show no change of shape. Occasionally a specimen would strike the acid with such momentum that it would be carried into it. When they had thus entered the acid, globules would appear about the sides of the anterior end as if the ciliated pits were discharging droplets of mucus. When such specimens escaped from the

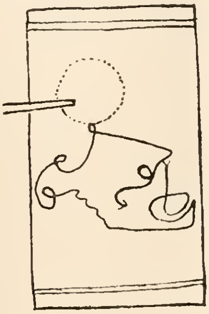

FIG. 3.

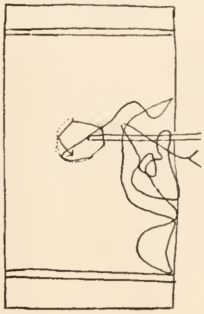

FIG. 4.

FIG. 3. Diagram indicating the path of a specimen when placed in eosin water and encountering a drop of 1/25 per cent. acetic acid solution. The dotted line indicates the contour of the enclosed drop of 1/25 per cent. acetic acid surrounded by eosin water.

FIG. 4. Diagram indicating the path of a specimen when placed in .1 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the enclosed drop of fresh water surrounded by .1 per cent. salt solution.

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[1] Fresh water in which just enough eosin was dissolved to make it distinguishable from the acid solution.

acid they ceased to show any exploratory movements. Perhaps this loss of the exploratory movements may be accounted for by injury of the ciliated pits. Usually the specimens would be unable to free themselves from the acid and would die. A 1/50 per cent. acetic acid solution was tried with similar results.

Thus it is seen that both 1/25 per cent. or 1/50 per cent. acetic acid are dangerous—even fatal media to *Microstoma*. Therefore .1 per cent. and .05 per cent. common salt solutions were tried. The first experiment was made upon an animal *seventeen hours* after removal from the pond. The animal was placed in a .1 per cent. salt solution and left to find the drop of fresh water which was placed in the center. The animal's course is indicated in text-figure 4. When the animal entered the fresh water it continued straight across it until it came to the far side of the drop, then it would turn away from the ciliated pit lying nearest the salt solution. By repeating this reaction to contacts with the salt solution the specimen would rotate in the drop of fresh water.

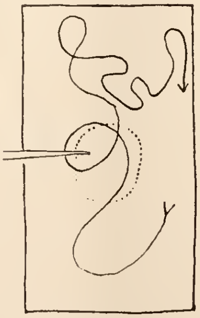

FIG. 5. Diagram indicating the path of a specimen when placed in .1 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the enclosed drop of fresh water surrounded by .1 per cent. salt solution.

We made only one experiment within twenty-four hours after the specimens were removed from the pond. *Five days later* specimens were taken from the same aquarium which furnished the last one. These specimens showed no definite reaction to the fresh water when they were treated like the specimen referred to in text-figure 4. The course of one of these is shown in text-figure 5. It can be seen that the animal passed through the fresh water twice and did not react to it at all.

These experiments suggested to us that a change may have taken place in the physiological condition of *Microstomas* which were kept in the laboratory for a certain length of time.

Collections were then made of *Microstomas* and 150 additional experiments performed during five months in order to test the suggestion made by these two sets of experiments.

These experiments must be divided into two groups and each of these groups into two sub-groups:

1. Reactions of *Microstomas* kept in the laboratory for less than twenty-four hours.

(*a*) When they were placed in fresh water and left to come in contact with an undisturbed drop of .05 per cent. common salt solution which was placed in the center.

(*b*) When they were placed in .05 per cent. salt solution and left to come in contact with an undisturbed drop of fresh water which was placed in the center.

2. Reactions of *Microstomas* kept in the laboratory for more than twenty-four hours.

(*a*) When they were placed in fresh water and left to come in contact with an undisturbed drop of .05 per cent. common salt solution which was placed in the center.

(*b*) When they were placed in .05 per cent. salt solution and left to come in contact with an undisturbed drop of fresh water which was placed in the center.

The following are three experiments of each of the above groups picked at random from our notebooks:

Group 1-*a* (specimens placed in fresh water and allowed to encounter a drop of .05 per cent. salt solution).

January 13, 1912. A specimen that had been in the laboratory *twenty hours* came in contact with the drop of .05 per cent. salt solution and each time reacted by turning away from the margin of the salt solution (text-figure 6-*a*).

February 3, 1912. A specimen that had been in the laboratory *eighteen hours* came in contact with the drop of .05 per cent. salt solution five times and each time it reacted by turning away from the margin of the salt solution (text-figure 6-*b*).

November 30, 1911. A specimen that had been in the laboratory *nineteen hours* came in contact with the drop of .05 per cent. salt solution six times and each time it reacted by turning away from the margin of the salt solution (text-figure 6-*c*).

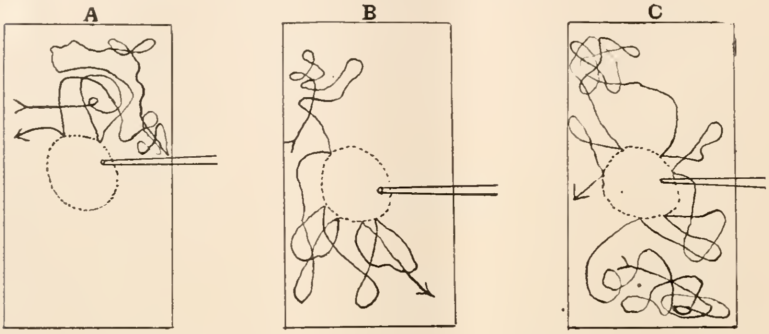

FIG. 6. Diagrams indicating the paths of specimens when placed in fresh water and encountering a drop of .05 per cent. salt solution. The dotted line indicates the contour of the drop of .05 per cent. salt solution surrounded by fresh water.

None of these specimens so treated entered the salt solution.

Group 1-*b* (specimens placed in .05 per cent. salt solution and allowed to encounter a drop of fresh water).

January 9, 1912. A specimen that had been in the laboratory *seventeen hours.* This one was caught in the drop of fresh water as the water was issuing from the pipette. Although it came in contact with the margin of the salt solution six times it did not leave the fresh water as shown by text-figure 7-*a*.

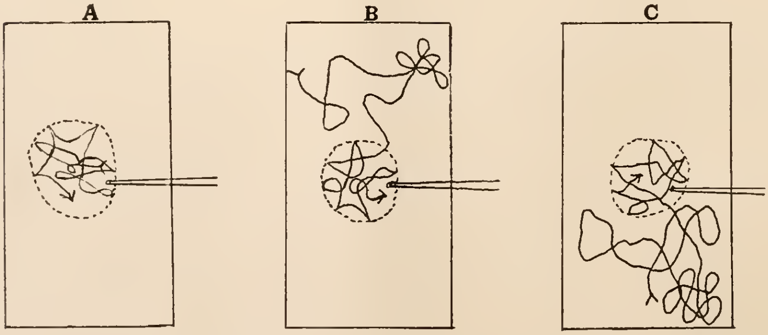

FIG. 7. Diagrams indicating the paths of specimens when placed in .05 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the drop of fresh water surrounded by .05 per cent. salt solution.

December 2, 1911. A specimen that had been kept in the laboratory *twenty hours* after entering the drop of fresh water did not leave it although it came in contact with the margin of the salt solution six times (text-figure 7-*b*).

January 13, 1912. A specimen that had been kept in the laboratory *twenty-four* hours after entering the drop of fresh water did not leave it although it came in contact with the salt solution six times (text-figure 7-*c*).

None of these specimens after entering the fresh water left it.

Group 2-*a* (specimens placed in fresh water and allowed to encounter a drop of .05 per cent. salt solution).

November 22, 1911. A specimen that had been kept in the laboratory *five days* passed five times from the fresh water into the salt solution and did not show any reaction to the latter at all (text-figure 8-*a*).

November 20, 1911. A specimen that had been kept in the laboratory *six days* passed from the fresh water into the salt solution three times and did not show any reaction to the latter (text-figure 8-*b*).

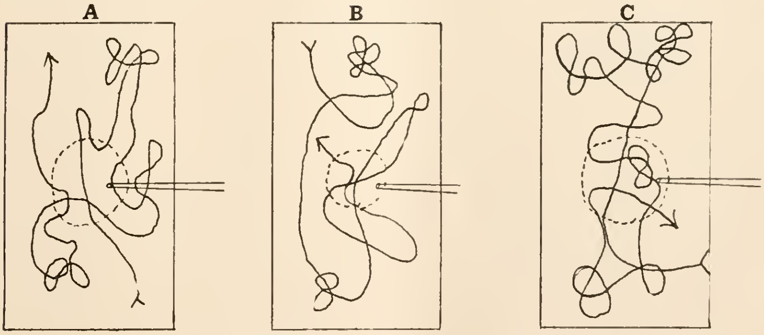

FIG. 8. Diagrams indicating the paths of specimens when placed in fresh water and encountering a drop of .05 per cent. salt solution. The dotted line indicates the contour of the drop of .05 per cent. salt solution surrounded by fresh water.

December 2, 1911. A specimen that had been kept in the laboratory *three days* passed from the fresh water into the salt solution three times and did not show any reaction to the latter (text-figure 8-*c*).

These specimens show a marked difference in their conduct as compared with the specimens of Group 1-*a*, in that they *pass through the drop of .05 per cent. salt solution* instead of turning away from it.

Group 2-*b* (specimens placed in .05 per cent. salt solution and allowed to encounter a drop of fresh water).

December 16, 1911. A specimen that had been kept in the laboratory *three days* entered the fresh water three times but each time it left it without showing any reaction to the salt solution (text-figure 9-*a*).

January 9, 1912. A specimen that had been in the laboratory *four days* entered the fresh water three times but each time it left it (text-figure 9-*b*).

November 20, 1911. A specimen that had been in the laboratory *five days* entered the fresh water three times but each time left it (text-figure 9-*c*).

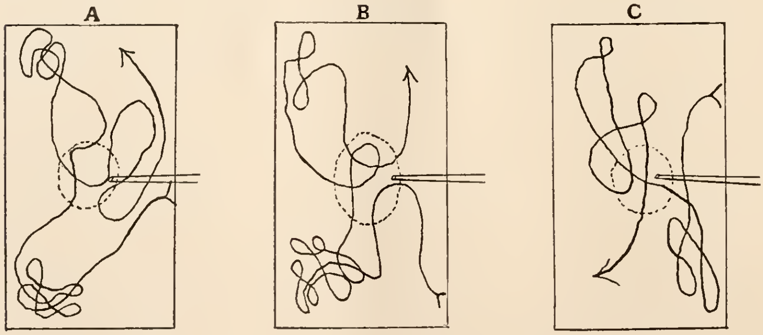

FIG. 9. Diagrams indicating the paths of specimens when placed in .05 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the drop of fresh water surrounded by .05 per cent. salt solution.

These specimens, too, show a marked contrast with Group 1-*b* in that *they pass indifferently from .05 per cent. salt solution into fresh water and back into the .05 per cent. salt solution* instead of remaining in the drop of fresh water.

Thus our experiments have sustained the inference that the physiological condition of *Microstoma* is lowered by the conditions peculiar to laboratory aquaria. This loss of physiological tone generally takes place after the first twenty-four hours.

The conditions most strikingly peculiar to laboratory aquaria are: (*a*) Radical changes of temperature; (*b*) rapid accumulation of bacteria. That the first is not the greater factor is shown by the fact that in one aquarium, in which for some reason bacteria did not accumulate, specimens remained from December 8 until February 3 and still gave reactions which showed that they had not lost their physiological tone. Text-figure 10-*a* indicates the path of such a specimen when placed in fresh pond water containing near its center a drop of eosin .05 per cent. salt solution. It would be well to compare this figure with text-figure 10-*b* which indicates the path of a specimen, with reference to a drop of .05 per cent. salt solution, which had been kept in an aquarium only five days, but upon which vessel a thin glea of bacteria had collected. As can be seen the latter specimen was indifferent to the salt solution.

This observation led us to test the reactions of two lots of specimens taken from fresh aquaria:

The first lot we placed in a watch glass with some of the brown glea of an old aquarium for two hours. These specimens showed plainly that they had lost their physiological tone. Text-figure 11-*a* indicates the path of one of these specimens when placed in .05 per cent. salt solution containing a drop of fresh water in the center. It can be seen that the specimen passed from one medium into the other six times and did not react to them at all.

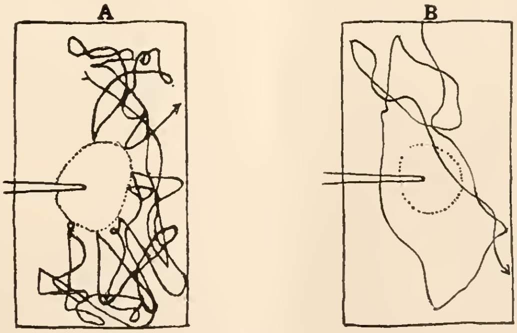

FIG. 10. Diagrams indicating the paths of specimens when placed in fresh wate and encountering a drop of .05 per cent. salt solution. The dotted line indicates the contour of the drop of .05 per cent. salt solution surrounded by fresh water.

The second lot we placed in a watch glass and then put this on top of a paraffin bath which kept the water at a constant temperature of 86° F. Although these specimens were kept at this high temperature for twenty-four hours they gave us reactions which showed that they had not lost their physiological tone.

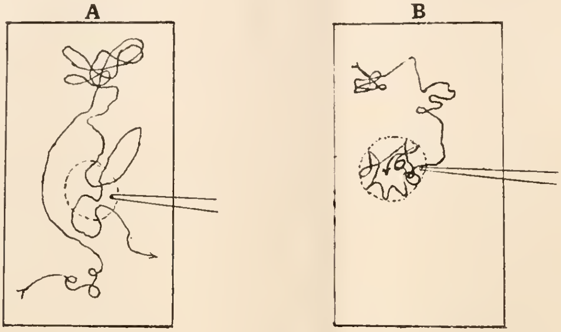

FIG. 11. Diagrams indicating the paths of specimens when placed in .05 per cent. salt solution and encountering a drop of fresh water. The dotted line indicates the contour of the fresh water surrounded by .05 per cent. salt solution.

Text-figure 11-*b* shows the path of one of these when treated like the one placed in bacteria. As soon as this specimen entered the fresh water it remained there by continually reacting to the salt solution. We watched such a specimen remain in the drop of fresh water for thirty minutes and then stopped observations because the salt solution began to diffuse into the fresh water. A comparison of text-figures 11-*a* and 11-*b* shows the difference between the reactions of the above two lots of specimens.

From these experiments we conclude that:

(*a*) *Microstomas* lose their physiological tone chiefly from the toxins thrown off by the bacteria.

(*b*) If change of temperature is a factor in this loss of physiological tone it is a very small one and they in time become adjusted to the new factor in their environment.

This change of physiological condition is somewhat analogous to the different physiological states found in the flat-worm Planaria by Pearl ('03) and summarized by Jennings in his "Behavior of the Lower Organisms" (p. 253, '06).

Finally a second class of experiments was carried out in order to test the value of the bilateral arrangement of the ciliated pits.

With a fine knife, made from a flattened needle, the ciliated pit was cut from either side of the animal. When the right pit was removed the specimen moved about in a spiral path keeping the remaining or left ciliated pit directed towards the center of the spiral path. When the right pit was left intact and the left destroyed the spiral movement was in the opposite direction. Thus the bilateral disposition of the ciliated pits of *Microstoma* serves the purpose of orienting or directing the *Microstoma* in its course through the water.

Again both pits were removed. In such cases it sometimes happened that the cut was a clean one, leaving the specimen divided into a minute, anterior part bearing the ciliated pits and a large, posterior part lacking the ciliated pits and the "brain." In such a case the minute portion moved in a highly active manner, tumbling about in all directions, while the large portion moved slowly in a direct line, except that a wide arc to the right or left was occasionally made. This direct course was continued until some inert object was encountered. Such contact would cause a change in the path of the specimen. This large part displayed no exploratory movements. So it is further suggested that the exploratory movements of *Microstoma* depend upon the ciliated pits being in a functional condition.

Summary.

1. There is present in the mid-ventral floor of the pharynx of *Microstoma caudatum* Ldy. a sensory epithelium, free from gland-ducts, which lies directly in contact with the posterior, transverse nerve commissure. The manner in which the pharynx behaves in testing food suggests that this is an elementary gustatory epithelium.

2. The ciliated pit has a glandular and a sensory region. Thus it resembles the "cerebral organs" of the Protonemertini and strengthens the affinity between the Rhabdocœles and the Nemertini.

3. *Microstoma caudatum* Ldy. living in its normal medium tests the surrounding water, etc., with its pharynx and ciliated pits. This testing is facilitated by making numerous exploratory movements with its anterior end.

4. We can recognize two physiological conditions in *Microstoma caudatum* Ldy.: 1st, when it has its physiological tone; 2d, when it does not have its physiological tone. In the first case it can and does distinguish between its normal medium and an artificial one such as .05 per cent. salt solution. In the second case it does not make this distinction.

5. This loss of physiological tone under laboratory conditions is caused chiefly by the toxins thrown off by bacteria; if change of temperature is a factor in this loss, it is but a slight one and in time *Microstoma* adjusts itself to this change and regains its physiological tone.

6. The bilateral disposition of the ciliated pits serves to direct the animal in its movements.

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Explanation of Plate I.

Fig. 1. Sexually mature specimen seen from left side. The ovary with oöcytes and oögonia shown lying by the left side of enteron. *V*, vagina; *M*, mouth leading into pharynx; *CP*, ciliated pit. Scale, 1 millimeter equals 7.5 micra.

Fig. 2. Ciliated pit arising from the epidermis. *E*, epidermis; *FC*, cells at fundus of pit. ×1,500.

Fig. 3. Gustatory epithelium of pharynx. The basal ends of the cells lying directly upon the nerve commissure. *N*, part of the nerve commissure. ×1,500.

Fig. 4. Sagittal section of ciliated pit. *DN*, dorsal, lateral nerve upon which the basal ends of the sensory cells rest; *G*, glandular cells of fundus; *G′*, a glandular cell which presents a duct leading into lumen of the pit. ×1,500.

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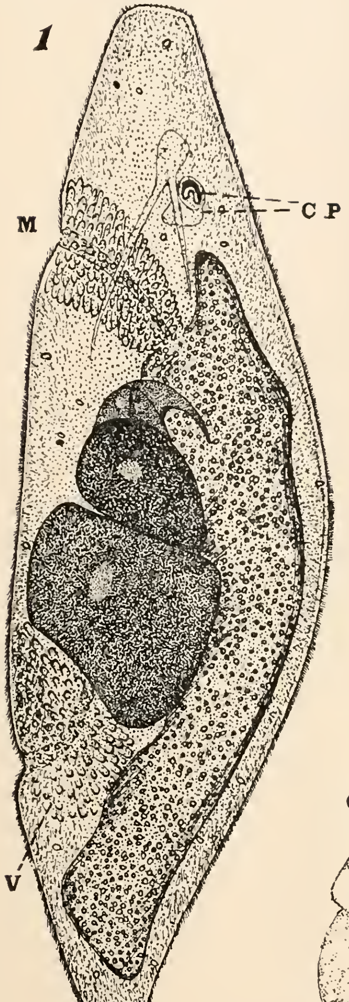

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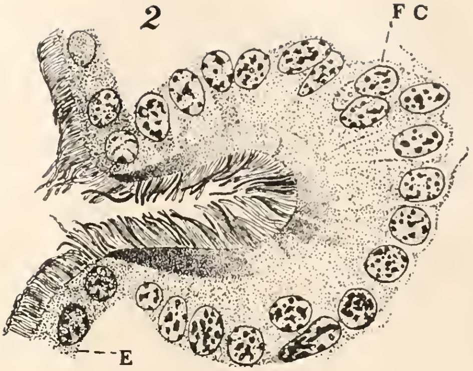

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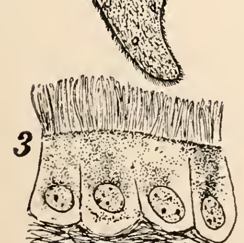

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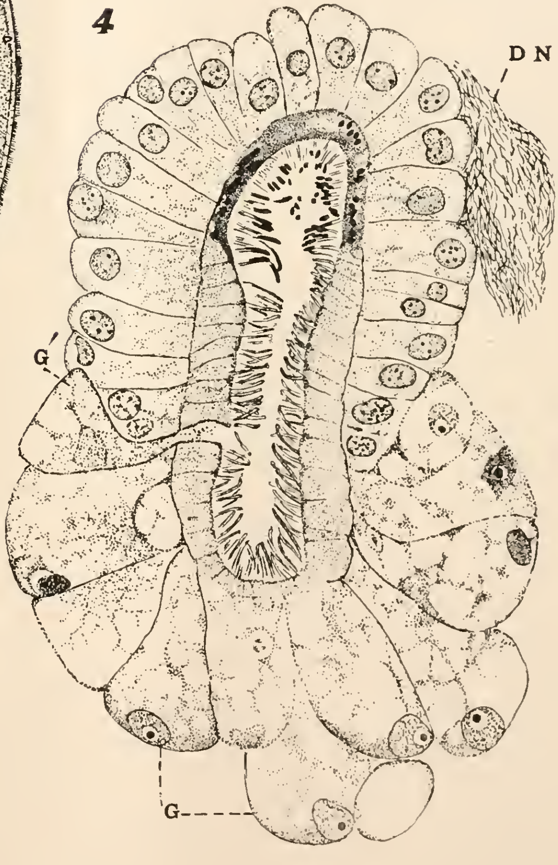

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BIOLOGICAL BULLETIN

ECOLOGICAL SUCCESSION.

IV. VEGETATION AND THE CONTROL OF LAND ANIMAL COMMUNITIES.

VICTOR E. SHELFORD.

		PAGE.
I.	Introduction	59
II.	Localities of Study	61
III.	Presentation of Data	65
IV.	Discussion of Data	72
V.	Causes of Succession or The Control of Animal Communities	73
	1. Materials for Abode and Food	73
	2. Soil	73
	3. Atmosphere	75
	(*a*) Temperature	76
	(*b*) Light	76
	(*c*) Combinations or Complexes of Factors	76
	(*d*) Evaporation in Forest Animal Habitats	79
	4. Influence of Physiography and Vegetation upon Animal Habitats.	82
	5. Stratifications of Condition	83
	6. Apparent Anomalous Distribution	84
	7. Agreement of Plant and Animal Communities	87
VI.	General Discussion	88
VII.	Summary	93
VIII.	Acknowledgments and Bibliography	94

I. INTRODUCTION.

In the preceding papers of this series, we have discussed the succession of animals in two types of aquatic habitats with particular reference to fish. While the data presented are only a minor part of those at hand they have served to illustrate some of the principles of succession in aquatic habitats. Discussion of other aquatic situations would enable us to point out many more important facts but we must now pass to land habitats. To illustrate principles here we might discuss the development of either forest or prairie animal communities on sterile mineral soil or in a filling pond. We have selected the succession of forest animal communities on sterile mineral soil and especially those on sand.

The forest conditions on the sand areas at the head of Lake Michigan were once among the best in North America for the study of the problems at hand and in spite of the fact that they are rapidly disappearing and have already been destroyed in some of the localities where the data here presented were collected, there are still various small areas between Indiana Harbor, Ind., and Sawyer, Mich., which taken together present the chief stages of forest development on sand. There are also localities outside the sand area, in which the later stages are to be found on other soils.

The searching of older literature, for possible statements which anticipate ideas here presented would require years and has not been undertaken in any adequate fashion as yet. Such anticipation of ideas and principles is perhaps to be expected but *organization and development are just at their beginnings.* On the plant side some older literature has been brought to attention. Buffon (1742) discovered that poplars precede oaks and beeches in the development of forest (Cowles, '11). Cowles found that in the Lake Michigan sand area cottonwoods precede pines, pines precede black oaks, black oaks precede red oaks, red oaks are usually followed by sugar maple and beech (Cowles '99, '01, '11; Clements, '05; Shantz, '06; Fuller, '11). We are to present certain representative facts concerning the development or succession of animal communities, accompanying and contributing to the causes of plant succession. The data presented are by no means complete as only a small part of the total number of animal species that might be collected from such a series of localities, has been studied. However the data are *adequate* for the purpose of illustrating principles and methods, and of bringing together some of the recent developments in the study of communities of organisms to focus them on the question as to the best method of obtaining and organizing the data of ecology of terrestrial animals.

II. Localities of Study.

The forest development series might be variously divided for purposes of study. The number of stages and variations that might be recognized is considerable. For the purpose just outlined we have selected five stages. Transition areas are present, but only the most important two, namely those between the first and second and the second and third, have been noted. The stages considered are (1) Cottonwood stage; (1–2) the transition between the cottonwoods and the pines; (2) pine stage; (2–3) transition between the pine and oak or mixed pines and oaks together with open places in the oak areas; (3) black oak stage; (4) red oak stage—the red oak associated with the black oak and white oak in the earlier stages and with the shag bark hickory in the later stages; (5) the beech and maple stage.

1. *Cottonwood Stage.*

The cottonwood (*Populus deltoides*) areas are located near the lake shore and the sand is always more or less shifting and rarely with more than traces of humus. The cottonwoods are usually small trees, scattered over the beach ridge, or the lakeward side of the shore dunes as the case may be. Between them are widely scattered bunches of grasses of which *Calamovilfa longifolia* is the most characteristic species and *Ammophila arenaria* usually common. Scattered individuals of *Artemisia canadensis* also occur. The shrubs, which are still more scattered or local, are the beach plum (*Prunus pumila*) and some of the xerophytic willows (*Salix glaucophylla*).

All localities are indicated on the map, p. 62, by letters used as designated below. Two principal cottonwood stations have been studied. One lies to the east, 1*A*, and one to the west, 1*B*, of Pine, Ind. At these points the cottonwood area is about ten rods wide and reaches inland just beyond the crest of the ridge where the plants of transition come in. (For the arrangement of ridges see Fig. 1, p. 137, of "Ecological Succession II.") Two other less fully studied areas have been visited frequently, viz., 1*C*, at Miller, Ind., and 1*D*, at Dune Park. (See map.)

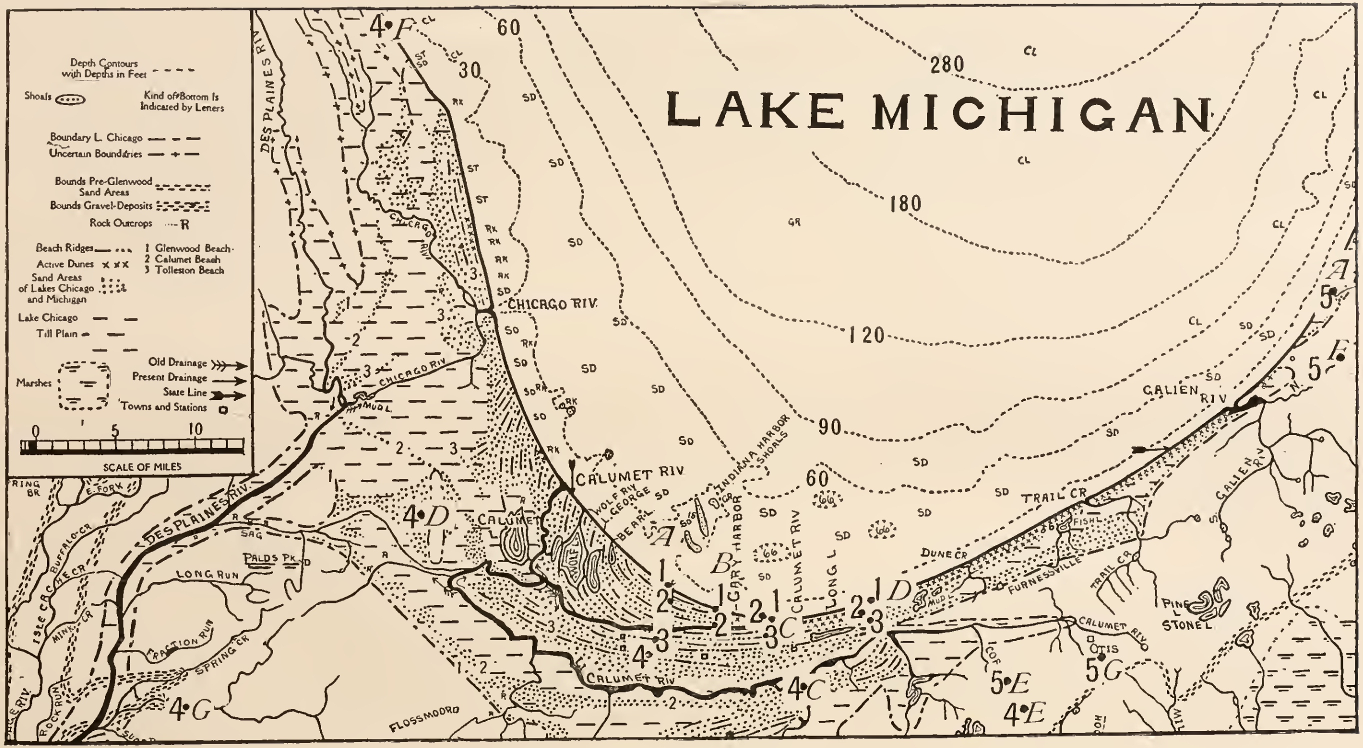
LAKE MICHIGAN
280
180
120
90
60
30
Depth Contours with Depths in Feet
Shoals
Kind of Bottom Is Indicated by Letters
Boundary L. Chicago
Uncertain Boundaries
Bounds Pre-Glenwood Sand Areas
Bounds Gravel-Deposits
Rock Outcrops R
Beach Ridges
1 Glenwood Beach
2 Calumet Beach
3 Tolleston Beach
Active Dunes
Sand Areas of Lakes Chicago and Michigan
Lake Chicago
Till Plain
Marshes
Old Drainage
Present Drainage
State Line
Towns and Stations
SCALE OF MILES
0
5
10
CHICAGO RIV.
CALUMET RIV
GALIEN RIV
DES PLAINES RIV
TRAIL CR
DUNE CR
CALUMET RIV
LONG L
GARY HARBOR
INDIANA HARBOR SHOALS
WOLF RIV
GEORGE
BERRY L
WOLF L
CALUMET
PALOS PK.
LONG RUN
SAG
MUD L
FLOSSMOORD
SPRING CR
FRACTION RUN
MINK CR
BUFFALO CR
ISLE CA
ROCK RUN
E. FORK
FURNESSVILLE
FISH L
PINE
STONE L
OTIS
4•F
4•D
4•G
5•E
4•E
5G
1 D
3
A
B
C
4•C
5
SD
CL
ST
RK
GR

FIG. 1. Map of the area of study. The stations are shown by small dots near the figures. The figures refer to forest stages as follows: 1, cottonwood stage; 2, pine stage; 3, black oak stage; 4, red oak stage; 5, beech stage. The letters standing near are for convenience in reference. *A–D* refer to stages on sand; *E–G* on clayey soil. Fuller has studied evaporation at stations 1, 2 and 3*D* and 5*G* and in the red oak stage at Palos Park.

1–2. *Mixed Pine and Cottonwood Stage.*

These areas lie on the first ridge from the lake. The sand shows traces of humus which is indicated by a slight darkening. The characteristic grass is the bunch grass (*Andropogon scoparius*) which occurs in dense bunches much more closely set than *Calamovilfa* in the preceding. The juniper (*Juniperus communis*), the bear berry and young pines (*Pinus Banksiana*) are the dominant plants, although old cottonwoods and scattered individuals of all the plants of the preceding stage are present. Two such stages were studied.

2. *Pine Stage.*

The sand here is stable and considerably blackened by humus except in blowouts where the wind keeps it constantly shifting. The trees are scattered stunted pines (*Pinus Banksiana*). The large areas of sand are covered with the recumbent bear berry; scattered specimens of many of the herbaceous plants of the earlier stages are to be found. The New Jersey tea (*Ceanothus americanus*) and the juniper are among the most characteristic shrubs. The cactus (*Opuntia Raffinesquii*) occurs in the older stages only. Three stations have been studied; two, 2*A*, and 2*B* (adjoining 1*A* and 1*B*) and 1*D*, at Dune Park, Ind.

2–3. *Mixed Pines and Black Oaks.*

The transition area between pine and oak is characterized by the presence of seedlings of the oak, the choke cherry, the fragrant sumac and an abundance of cacti and Liatris. The ground is much darkened. Herbs are much more numerous than earlier but bare spots of relatively clear sand continue, even after the oaks have entirely displaced the pines. Such transition areas proper have been so much disturbed that only the open bare sandy places in the oak areas are here considered as belonging under this head.

3. *Black Oak Stage.*

We now find the sand to be much darkened by humus and locally covered with a dry moss or with dead leaves. Grasses also partly cover the ground. The shrubby undergrowth is made up of blueberry, choke cherry and New Jersey tea. The oaks (*Quercus velutina*) constitute a much thicker stand than the pines but the type which we are considering here has the open places grassy and covered with a growth of vetch (*Tephrosia virginiana*), golden-rod and other Compositæ. The chief points of study were 3*A*, at Miller, Ind., 3*B*, near Clark, Ind., and 3*C*, at Dune Park.

4. *Red Oak Stage.*

This was originally the most abundant type of forest near Chicago but areas which have not been disturbed by man are few in number. On the sand, hickories are rare. The forest is usually made up of black oak, red oak, and white oak. This is true at 4*A*, 4*C*, and 4*D*, of the map. The more mesophytic type is made up of white oak, red oak, and hickory, with the red oak and the hickory dominating. Our type four then, represents a range of conditions. All the stages are included here and all are characterized by the absence of bare sand and other mineral soil, and by the presence of a carpet of leaves and humus which covers the ground. There is a well marked shrubby and herbaceous growth. The characteristic shrubby species, are blueberry, *Viburnum*, *Cornus*, and *Crataegus*. The shrubs are usually quite numerous and make thick stands locally in the forest. The places studied are, 4*A*, at Hessville, Ind., 4*C*, at Liverpool, Ind. and 4*D*, at Beverly Hills in Chicago. These represent white oak, red oak, and black oak on sand. 4*E*, 4*F* and 4*G*, represent areas on till clay which are in something like primeval conditions.

5. *Beech Stage.*

This type is characterized by beech and sugar maple. The mineral soil is covered with several centimeters of humus and a very thick layer of leaves which is often matted together by fungus hyphæ. The number of species of trees is smaller but the number of species of small shrubs is greater than in the preceding stage. The number of individual shrubs however is smaller. Here the trees close the over-head spaces and make a dense shade, while the lower forest is open. The localities of study are, 5*A*, at Sawyer, Mich., on sand, 5*C*, at Woodville, Ind., now destroyed, 5*F*, at Sawyer, Mich., and 5*G*, at Otis, Ind., on clay

III. Presentation of Data.

We will begin with the animals of the earliest forest stage and proceed to those of the latest. For purposes of comparison animals must be divided into groups with comparable habitat relations. Warming's ('09, p. 138) division into strata may be employed (see Dahl, '08, p. 11). In any locality we note that there are several levels which may be occupied by animals. Some animals live below the surface of the ground and constitute the *Subterranean stratum.* Others live at the surface of the ground and constitute the *Ground stratum.* Animals inhabiting the herbaceous vegetation, and low shrubs, etc., make up the *Field stratum.* Those living on the shrubs and young trees make up the *Shrub stratum*, and those on the trees, the *Tree strata.* Such a division is essential to any comparison of the animals of different forests, steppes, etc. The ground stratum of one cannot well be compared with the field stratum of another; like strata must be compared.

Many animals invade several strata in connection with their various activities. They should, however, be classed primarily in the stratum in which they breed and secondarily in the stratum or strata in which they feed or forage. The breeding activities are of especial importance to the animals in question, while the feeding and foraging influence other animals. The study of the lower strata of the forest presents no difficulties. The tree stratum however is usually far enough above the ground to make observation difficult. The data at hand are rather incomplete at this point and accordingly the discussion is confined to the lower strata.

Tables I. and II. and the lists following them show the distribution of about 200 species of animals in the forest stages. These tables and lists include chiefly animals that have been encountered in these situations often during several seasons. Where collections are known not to have been representative, they are omitted. For example most of the Diptera collected were pre-

TABLE I.

Showing the distribution of the animals belonging to the subterranean and ground strata of two or more of the animal communities of the forest stages indicated by numbers:—1, cottonwood stage; 1–2, mixed pine and cottonwood; 2, pine stage; 2–3, mixed pine and black oak stage and open places in the black oak forest; 3, the black oak stage, in the later stages white oak occurs; 4, stages containing red oak but not beech and maple (the earlier stages are black oak, white oak and red oak and the later stages white oak, red oak and hickory); 5, beech and maple stage which usually contains some basswood. In the numbered columns, the star indicates that a species is present, *F* signifies few, *C* common, *A* abundant. Strata are indicated by letters at the heads of the columns:—*a*, subterranean including rotten logs; *b*, ground; *c*, vegetation; in these columns *F* indicates feeding place; *B* indicates breeding place. Letters in column marked *lit.* refer to literature in the special bibliography.

Common Name.	Scientific Name.	1	1–2	2	2–3	3	4	5	*a*	*b*	*c*	*lit.*
Tiger beetle	*Cicindela lepida* Lee	*C*	*F*						*B*	*F*		*A*
Sand spider	*Trochosa cinerea* Fab.	*C*							*B*	*B*		*B*
Maritime grasshopper	*Trimerotropis maritima* Harr.	*C*	*F*						*B*	*F*		*C*
Tiger beetle	*Cicindela formosa generosa* Dj.	*F*	*C*	*F*					*B*	*F*		*A*
Long-horned grasshopper	*Psinida fenestralis* Serv.	*C*	*C*	*C*	*C*				*B*	*F*		*C*
Burrowing spider	*Geolycosa pikei* Marx.	*C*	*C*	*C*	*C*				*B*	*F*		*B*
Bembex	*Microbembex monodonta* Say	*C*	*C*	*C*	*C*				*B*	*F*	*F*	*C*
Bembex	*Bembex spinolæ* Lep.	*F*	*C*	*C*	*C*				*B*	*F*	*F*	*D*
Termite	*Termes flavipes* Koll.	*C*	*F*	*F*	*F*	*F*			*B*			*E*
Wasp	*Dielis plumipes* Dru.		?	*	*				*B*	*F*	*F*	*F*
Digger wasp	*Anophilus divisus* Cress.		*C*	*C*					*B*	*F*	*F*	*D*
Sand locust	*Agenotettix arenosus* Han.		*	*					*B*	*F*		*C*
Ant	*Lasius niger americanorum* Em.		*C*	*F*					*B*	*F*	*F*	*I*
Mottled sand locust	*Spharagemon wyominganum* The.		*C*	*C*	*C*				*B*	*F*		*C*
Migratory locust	*Melanoplus atlanis* Ril.		*C*	*C*	*C*				*B*	*F*		*C*
Tiger beetle	*Cicindela scutellaris Lecontei* Hald.		*F*	*C*	*C*				*B*	*F*		*A*
Locust	*Melanoplus angustipennis* Dod.		*C*	*C*	*C*				*B*	*F*	*F*	*C*
Sixlined lizard	*Cnemodophorus sexlineatus* Linn.		*F*	*C*	*C*				*B*	*F*		*H*
Andrenid	*Auglochlora confusa* Rob.			*	*				*B*	?	*F*	*J*
Parasitic bee	*Epeolus pusillus* Cress.			*	*				?	?	*F*	*J*
Burrowing bee	*Spechodes dicroa* Sm.			*	*				*B*	*F*	*F*	*D*
Ammophila	*Ammophila procera* Klg.			*F*	*C*	*F*			*B*	*F*	*F*	*D*
Ant lion	*Mymeleon* sp.?				*	*				*B*		

TABLE I (*continued*).

Common Name.	Scientific Name.	1	1–2	2	2–3	3	4	5	*a*	*b*	*c*	*lit.*
Flat larvæ	*Pyrochroidæ*			*F*	?	*F*	*C*	*C*	*B*			*L*
Snails	*Zonites arborens*			*F*	*F*	*F*	*C*	*F*	*B*			*P*
Centipede	*Lithobius* sp.			*F*	?	*F*	*C*	*C*	*B*			
Eyed elater	*Alaus oculatus* Lin.					*F*	*C*	*C*	*B*			*L*
Borer	*Orthosoma brunneum* Forst.					*F*	*C*	*C*	*B*			*L*
Tree frog	*Hyla pickeringii* Hol.					*F*	*C*	*C*		*F*		*M*
" "	*Hyla versicolor* Lec.					*C*	*F*			*F*		*M*
Snail	*Polygyra thyroides* Say					*F*	*C*	*F*	*B*	*F*	*F*	*N*
Sow bug	*Porcellio rathkei* Brandt					*F*	*C*	*C*	*B*			*O*
Snail	*Pyramidula alternata* Say						*C*	*C*	*B*	*F*		*P*
Snail	*Circinaria concava* Say						*C*	*C*	*B*	*F*		*P*
Sow bug	*Cyclisticus convexus* De G.						*	*	*B*	*F*		*O*
Centipede	*Lyasopetalum lactarium* Say						*	*	*B*			*Q*
Centipede	*Geophilus rubens* Say						*	*	*B*			*Q*
Millipede	*Fontaria corrugate* Wood						*C*	*F*	*B*			*Q*
Millipede	*Spirobolis marginatus* Say						*C*	*C*	*B*			*Q*
Slug	*Philomycus carolinensis* Bosc.						*C*	*C*	*B*	*F*		*P*
Snail	*Pyramidula solitaria* Say								*B*	*F*		*O*
Beetle	*Galerita janus* Fabr.						*C*	*C*	*B*	*F*		*L*
Melandrydæ	*Penthe promelia* Fabr.						*C*	*	*B*			*L*
Burying beetle	*Silpha surinamensis* Fabr.						*C*	*C*	*B*	*F*		*L*
Harvestman	*Liobromum nigripalpi*						*	*				
Ant	*Camponotus hereculeanus pennsylvanicus* De G						*C*	*C*	*B*	*F*	*F*	*I*
Beetle	*Cerchus piceus* Web.							?	*B*	*F*		*L*

served in alcohol and only a few of these could be identified. The flies from one station were pinned and accordingly were all identified so that the inclusion of these in the tables and lists would introduce error. The lists and tables are divided into two groups; the first includes the inhabitants of the ground and subterranean strata, the second of the field, shrub and tree strata.

List of Animals Recorded in the Ground and Subterranean Strata of the Stages Indicated only.

In the first column are common names and in the second scientific names. In the third column *B* indicates breeding; *F*, feeding; *H*, hibernating of animals on the situation indicated in column 4 following. Letters in column *lit.* refer to literature cited in the special bibliography at the end of the paper. Statements made on the authority of others are in italics, those starred are by A. B. Wolcott.

1	2	3	4	*lit.*
	Pine Stage.			
Bee (Andrenidæ)	*Halictus melumboni* Rob.	*B?*		*J*
Larridæ	*Tachytes texanus* Cres.			*F*
Scoliidæ	*Plesia interrupta* Say			
Ampulicidæ	*Anophilus marginatus* Say		In sand	*F*
Beetle (Elateridæ)	*Cardiophorus cardisce* Say		On sand	*L*
Blue racer	*Coluber constrictor* Lin., Var.	*B*	In sand	*R*
Ground squirrel	*Spermophilus 13-lineatus* Mitch.	*B*	In sand	*S*
Elateridæ	*Alaus myops* Fabr.	*B?*	Under pine bark	
	Black Oak Stage.			
Elateridæ	*Lacon rectangularis* Say	*B*	Under Opuntia	*
Erotylidæ	*Languria trifasciata* Say	*B*	Under Opuntia	*
Coral winged locust	*Hippiscus tuberculatus* Beau.	*B*	In sand	*C*
Parasitic bee	*Cœlioxys rufitarsus* Smith	*B*	Bee nest	*R*
Psithyridæ	*Psithurus* sp.			
Eumenidæ	*Odynerus anornis* Say			
Hog-nosed snake	*Heterodon platirhinos* Latr.	*B*	In sand	*R*
	Red Oak Stage.			
Green tiger beetle	*Cicindela sexguttata* Fabr.	*B*	In soil	*A*
White-faced hornet	*Vespa maculata* Lin.	*H*	Rotten wood	*G*
Andrenidæ	*Augochlora pura* Say			
Ant	*Lasius umbratis mixtus aphidicola* Walsh	*B*	Log	*H*
Ant	*Camponotus ligniperda* Latr. *noveboracensis* Fitch	*B*	Log	*H*
Ground beetles	*Pterostichus sayi* Brulle	*B*	Rotten log	*L*
Tenebrionidæ	*Meracantha contracta* Beau.	*B*	Rotten log	*L*
Tenebrionidæ	*Uloma impressa* Mels.	*B*	Rotten log	*L*
Scarabæidæ	*Geotrupes splendidus* Fabr.	*B*	Rotten log	*L*

Staphylinidæ	*Staphylinus violaceus* Grav.	*B*	Rotten log	*L*
Elateridæ	*Melanotus communis* Byl.		Rotten log	*L*
Slug	*Pallifera dorsalis* Bin.	*B*	Log	*P*

Beech Stage.

Frog	*Rana sylvatica* Le Conte	*F*	Ground	*M*
Salamander	*Plethodon cinereus* Gr.	*BF*	Under leaves	*H*
Snail	*Polygyra inflecta* Say	*BF*	Leaves and log	*P*
Snail	*Polygyra oppressa* Say	*BF*	Leaves and log	
Snail	*Polygyra fraudulentia* Pil.	*BF*	Leaves and log	*P*
Snail	*Polygyra palliata* Say	*BF*	Leaves and log	
Snail	*Pyramidula perspectiva* Say	*BF*	Leaves and log	*P*
Snail	*Pyramidula solitaria* Say	*BF*	Leaves and log	*P*
Ground beetle	*Pterostricus corecinus* Newm.	*BF*	Leaves and log	*L*
Beetle	*Xylopodus saperaioides* Oliv.	*B*	Under bark	*LV*

The distribution of the animals of the field shrub and lower tree strata are shown in Table II. and the lists which follow.

TABLE II.

Showing the distribution of animals recorded from the vegetation in more than one of the animal communities of the forest stages indicated by numbers: 1, the cottonwood stage; 1–2, mixed cottonwood and pine stage; 2, pine stage; 2–3, mixed pine and oak stage and open places in the oak forest; 3, black oak stage, in its later phases white oaks occur; 4, stages containing red oak but not beech and maple; 5, beech and maple stage.

Common Name.	Scientific Name.	1	1–2	2	2–3	3	4	5
(*a*) Spider (Thomisidæ)	*Philodromus alaskensis* Key	*	*	*				
(*b*) Butterfly	*Anthocharis genuita* Fabr.		*	*	*			
(*c*) Spider (Epeiridæ)	*Epeira domocilorum* Hentz		*	?	?	?	*	*
(*dd*) Dusky plant bug	*Lygus pratensis* Lin.			*	*			
(*d*) Phasmidæ	*Diaphoromera femorata* Say.					*	*	
(*e*) Assassin bug	*Diplodus* sp.					*	*	*
(*f*) Spider (Thomisidæ)	*Misumessus asperatus* H		*			*	*	*
(*ff*) Spider (Dictynidæ)	*Dictyna foliacea* Hentz.					*	*	*
(*g*) Spider (Epeiridæ)	*Epeira gigas* Leach						*C*	*F*
(*h*) Spider (Theridiidæ)	*Theridium frondeum* Hentz						*	*
(*i*) Bug	*Acanthocephala terminalis* Dall						*	*
(*j*) Stink bug	*Nezara hilaris* Say						*	*
(*k*) Stink bug	*Podisus maculiventris* Say						*	*

The letters below at the left refer to the species opposite which they stand in Table II. and the numbers refer to the forest stages as at the heads of the columns of Table II. The capitals and italics have the same meaning as in the preceding table and list (Table I.).

a—from cottonwoods and juniper (1, 1–2, 3) (*B*, *K*).
b—from *Arabis lyrata* (*S*).
c—from pine and herbaceous vegetation (*B*) (4) (*K*).
d—from the trunks of various trees.
e—from black oak (3), red oak (4) and from maple (5).
f—Monarda (2–3) and black oak (3) maple (5) (*B*, *K*).
ff—*F* Monarda (3) (*K*).
g—from undergrowth (4) and beech (5) (*K*, *B*).
h—from shrubs (4) and young beech (5) (*B*, *K*, *T*).
i—shrubs (4) and maple trunk.
j—from red oak trunk (4) and beech trunk (5) (*Linden*, *Citrus*, *Gossypium U*).
k—? (4) and beech leaves (5) (*predaceous U*).
dd—herbs (*W*).

The food plant records in the literature are of no great significance ecologically because the character of the leaves of trees growing in open places and in forest and the physical conditions surrounding them are so different that a species feeding on a given tree in the forest might not feed on the same tree in the open and vice versa.

LIST OF ANIMALS RECORDED FROM THE FIELD, SHRUB, AND TREE STRATA OF THE FOREST STAGES NOTED.

All columns and symbols as in the list following Table I.

Cottonwood Stage.

1	2	3	4	*Lit.*
Chrysomelid beetle	*Disonycha quinquevittata* Say	*B*, *F*	Willow	*L*
Long-horned borer	*Plectodera scalator* Fab.	*B*, *F*	Cottonwood	*L*
Gall aphid	*Pemphigus populicaulis* Fitch	*B*, *F*	Cottonwood	*Z*
Gall aphid	*Pemphigus vagabundus* Walsh	*B*, *F*	Cottonwood	*Z*

Pine Stage.

1	2	3	4	*Lit.*
Leaf beetle	*Nodonota tristis* Oliv.	*F*	Herbs	*L*, *V*
	Bassareus lativittis Germ.	*F*	Herbs	
Spider (Thomisidæ)	*Xysticus formosus* Banks		Juniper	*R*
Spider (Attidae)	*Dendryphantes octavus* Hentz		Juniper	*Y*, *B*, *K*
Spider (Theridiidæ)	*Theridium spirale* Em.		Juniper	*B*, *K*
Engraver beetle	*Ips grandicollis* Eich.	*B*, *F*	Pine	*V*
Pitch moth	*Evetria comstockiana* Fern.?	*B*, *F*	Locust	*V*

Black Oak Stage.

1	2	3	4	*Lit.*
Thread-waisted wasp	*Harpactopus* sp.	*F*	Primrose	*D*
Andrenid	*Agapostemon splendens* Lepel	*F*	Primrose	*D*
Spider (Thomisidæ)	*Philodromus pernix* Black	*F*	Herbs	*K*
Spider (Epeiridæ)	*Argiope trifasciata* Forsk.	*F*	Herbs	*K*
Sprinkled locust	*Chlœaltis conspersa* Har.	*F*	Herbs	*C*
Grasshopper	*Schistocera rubignosa* Har.	*F*	Herbs	*C*

Tree cricket	*Œcanthus fasciatus* Fitch.	*B, F*	Herbs	*C*
Texas grasshopper	*Scudderia texensis* Scud.	*B, F*	Herbs	*C*
Cone-head grasshopper	*Conocephalus ensiger* Scud.	*B, F*	Herbs	*C*
Meadow grasshopper	*Xiphidium strictum* Scud.	*B, F*	Herbs	*C*
Stink bug	*Euschistus variolarius* Pal.	*B, F*	Herbs	*W*
Flower bug	*Tripleps insidiosus* Say.	*F*	Herbs	*T*
Fork-tailed larvæ	*Cerura* sp.	*B, F*	Cherry	*V*
Fulgorid	*Otiocerus degeeri* Kirby	*B, F*	Oak	*T*
Flat bug	*Neuroctenus simplex* Uhl.	*B, F*	Oak	*T*
Colydiid beetle	*Ditoma quadriguttata* Say.	*F*	Oak	*V*
Prominent larva	*Heterocampa guttivittata* Wlk.	*B, F*	Oak	*V*
Prominent larva	*Nadata gibbosa* S. and A.	*B, F*	Oak	*V*
Tree hopper	*Telemona querci* Fitch(*monticola*)	*B, F*	Oak	*T*
Coreidæ	*Chariesterus autumator* Fabr.	*B, F*	Oak	*T*
Jassid	*Typhlocyba querci* var. *bifasciata* Gall.	*B, F*	Oak	*T*
Jassid	*Phlepsius irroratus* Say.	*B, F*	Oak	*T*

Red Oak Stage.

Rove beetle	*Tachinus pallipes* Grav.	*B, F*	Mushrooms	*D*
Spider (Clubionidæ)	*Anyphæna conspersa* Key.	*F*	Herbs	*B, K*
Spider (Dictynidæ)	*Dictyna* sp. (juvenile)	*F*	Herbs	*B, K*
Spider (Attidæ)	*Mævia niger* Hentz	*F*	Herbs	*Y, B, K*
Locustidæ	*Atlanticus pachymerus* Burm.	*B*	Grass	*C*
Spider (Epeiridæ)	*Acrosoma gracilis* Wal.	*F*	Shrubs	*B*
Spider (Epeiridæ)	*Acrosoma spinea* Hentz	*F*	Shrubs	*B*
Spider (Clubionidæ)	*Clubiona* sp.		Shrubs	*B*
Spider (Epeiridæ)	*Mangora maculata* Key.		Shrubs	*K*
Jassid	*Scaphodius auronitens* Prov.		Shrubs	
Beetle	*Odontota nervosa* Panz.		Shrubs	
Bug (Nabidæ)	*Coriscus annulatus* Reut.	*B*	Shrubs	
Spider (Lyngyphiidæ)	*Linyphia phrygiana* Kock.		Shrubs	
Cicada	*Cicada linnei* S. and G.	*F*	Young maple	
Leaf beetle	*Calligrapha scalaris* Lec.	*B F*	Young maple	
Stink bug	*Euschistus tristigmus* Say.	*B F*	Young maple	
Arctiidæ	*Halisdota* sp.	*B F*	White oak	*B*
Oak worm	*Anisota senitoria* Sm. and Abb.	*B F*	White oak	*D*
White oak gall	*Andricus semiator* Harr.	*B F*	White oak	*Z*
Tree cricket	*Œcanthus angustipennis* Fitch.	*B F*	Red oak	
Katydid	*Cyrptöphyllus perspecivius* L.	*B* —	Red oak	
Leaf beetle	*Xanthonia 10-notata* Say.	*F*	Red oak	
Prominent larva	*Symmirista albifrons* S. and A.	*B* —	Maple	*V*
Prominent larva	*Datana anguisii* G. and R.	*B* —	Hickory	
Aph id	*Phylloxera caryæ-caulis* Fitch	*B* —	Hickory	*Z*

Beech Stage.

Beetle	*Boletobius cinctus* Grav.		Mushrooms	
Fungus beetle	*Boletotherus bifurcus* Fabr.		Shelf fungus	
Snout beetle	*Piazurus oculatus* Say.		Shrubs	

Cercopidæ (bug)	*Clastoptera obtusa* Say.	— *F*	Hic., map., hazel	*T*
Tettiginidæ (leaf hop)	*Gypona octolineata* Fitch.	— *F*	Hic., map., beech	*J*
Leaf hopper	*Jassus oblitururus* Say.	— *F*	Maple	
Ichneumonidæ	*Thalessa atrata* Fabr.	*B* —	Larvæ	*V*
Lace wing	*Crysopa rufialbris*	*B* —	Maple	
Lace bug	*Gargaphia tiliæ* Walsh.	*B* —	Beech	*V*
Ichneumonidæ	*Trogus vulpinus* Cb.	*B*	Larvæ	*D*
Pentatomidæ	*Banasa calva* Say.	*F*	Beech	
Lampyrid beetle	*Podabrus basilaris* Say.	——	Maple	*V*
Lycosidæ	*Wala mitrata* Hentz.		Maple	
Theridiidæ	*Notionella interpres* Cam.		Maple	
Lampyridæ	*Telephorus tubercolatus* Lec.		(*Alder.*)	*L*
Harvestman	*Oligolophus pictus* Wood		Maple trunk	—

IV. Discussion of Data.

An examination of Table I. and the lists of ground and subterranean animals, shows that we have on and under the ground a change in species as we pass from the youngest to the oldest stage of forest development. We note also, where data permit estimation of relative abundance, that as we pass from the youngest to the oldest stage, a species is first few in numbers, then common and later decreasing again. Examination of Table II. and the lists of animals inhabiting vegetation, shows the same phenomenon though the delimitation appears somewhat sharper, possibly because these animals are related to plants and the differences in physical conditions are accompanied by quite different plants.

We note that in general, with the change of conditions accompanying the development of forest upon sterile sand or other mineral soil there is also an almost complete change of animal species. This change is comparable to that associated with the development of a stream (Shelford, '11[1]) and the filling of a pond (Shelford, '11[2] and '11[4]). This change in *mores*, if viewed at the oldest point in the environmental series, is *ecological succession.* For example at station 5*A* where beech forest occurs on sand dunes the cottonwood community has probably been succeeded by the pine community; the pine community by the black oak community; the black oak community by the red oak community which has given way to the present beech community.

This could be discussed as in the cases of the ponds and streams. The discussions already published (l. c.) are sufficient to illustrate the methods and principles. Furthermore the succession of conditions and of the tiger beetles applying to forest development has already been briefly outlined (Shelford, '07). (See Adams, '08, '12.)

V. CAUSES OF ANIMAL SUCCESSION AND THE CONTROL OF ANIMAL COMMUNITIES.

1. The causes of plant succession as summarized by Cowles, '11, may be divided into those related to atmosphere and those related to soil. In the case of animals we recognize also difference in food and materials for abode. *Physical conditions* are believed to be *most* important, as indicated by the great mass of experimental work on animal *behavior*. Representative literature supporting this view is cited in the discussions which follow.

1. *Materials for Abode and Food.*

The former are of great importance. There are the greatest differences between the different forest stages, in this matter. The plants of the later stages are more numerous and the leaves less strongly cutinized, even when the plants belong to the *same* species. The difference in leaf structure may be a factor in limiting the distribution of the phytophaga to a certain part of the range of a species of plant. The leaves, fallen logs, and all conditions in which the animals make their abodes, change as the forest develops. Food has been but little studied and we know little or nothing as to what aspects of the food factor are important. Dahl ('96) has studied the relation of carrion eating animals to their food supply.

2. *Soil.*

Those causes of plant succession which are due to progressive changes of soil, may be briefly summarized from an inspection of the description of stations given above. The chief changes obvious to the eye are an increase of vegetation, of leaf covering and of humus.

(*a*) The last of these changes increases the water holding capacity of the soil, while the other two decrease the evaporation from the soil. The water holding power of different soils is different. It increases with the decrease in size of the soil particles and with the addition of humus which takes up water by imbibition. The amount of water in the soil is usually expressed in terms of per cent. of weight but a soil with 8 per cent. of moisture may not give up water to an organism as readily as another soil with only 2 per cent. It is necessary therefore, to determine the capacity of a soil to retain or give up moisture. This has been determined for a number of soils by Briggs and McLane ('07) and Briggs and Shantz ('12), in terms of what they call the moisture equivalent. The moisture equivalent of a soil is the percentage of water which it can retain in opposition to a centrifugal force 1,000 times that of gravity. This has been determined for a number of soils (l. c., '12, p. 57). The maintenance of turgor in plants is believed to be a purely physical matter. If the roots of a plant are in a mass of soil, the plant gradually reduces the water content until the permanent wilting occurs. The *wilting coefficient* of a soil is the moisture content (in percentage of dry weight) at the time when the leaves of the plant growing in the soil first undergo a permanent reduction in moisture content, as a result of a deficiency of moisture supply. The *moisture equivalent* of a soil is 1.84 times the *wilting coefficient for wheat*, used as a standard plant. Fuller ('12) states that the wilting coefficient of dune sand is about 0.75 per cent. while the usual moisture content of the cottonwood dune sand is two or three times this amount. For the clay soil of the oak-hickory forest, according to McNutt and Fuller ('12) the coefficient is about 8 per cent. These standards of soil moisture indicate the amount of water available to animals through direct contact with the soil or available for evaporation into the air of cavities which they construct for themselves beneath the surface of the soil. The soil inhabiting animals of the cottonwood area live in the presence of a greater amount of available water than do the animals of the oak hickory forest.

(*b*) *Plants and Animals.*—Cowles ('11) mentions the importance of soil bacteria which increase with the increase of the humus, and the development of substances toxic to the plants producing them (Schreiner and Reed, '07). Little is known of the effect of animals upon the soils in which they live but if excretory products ever accumulate in any quantity, they probably have a detrimental effect, especially upon the animals which produce them (Colton '08 and citations). On the other hand, many burrowing animals bury organic material and bring mineral soil to the surface. The digger wasps must add much to the sand by burying many insects for their young. Earth worms appear in the later stages and contribute to soil formation (Darwin). Cowles states further on the authority of Transeau that humus accumulation alters soil aeration.

(*c*) *Temperature.*—Transeau found that the temperature of bog soil and bog water is below that of other soils and waters. This has however not been observed in the case of dry soils. The differences between soil on the beach at Sawyer, Mich., Aug. 19, 1911, at 3.00 P.M. and in the beech woods near at hand was as follows: Air 20° C., upper one half inch of sand of cottonwood area 38°–39° C., sandy soil of beech woods 19°–20° C., a difference of 19° C. The upper one half inch of bare sand goes as high as 47° C. on the hottest days of summer while the soil in the beech woods is probably always a little cooler than the air at the time of the air maximum. Cottonwood soil temperature on the hottest summer days at about 3.00 P.M. has been found to be as follows:

TABLE II.

SHOWING VARIATION OF SAND TEMPERATURE WITH DEPTH AND MOISTURE CONTENT.
AIR 36° C.

	Dry Sand.	Moist Sand
1.25 cm. below surface	47° C.	32° C.
3–4 cm. below surface	38° C.	31° C.
8–9 cm. below surface	35° C.	29° C.
10–11 cm. below surface	33° C.	——
12–13 cm. below surface	32° C.	27° C.
17–18 cm. below surface	30° C.	——

Simultaneous readings in later forest stages were impracticable. Even where exposed to the sun moist sand is kept at a lower temperature by the evaporation.

3. *Atmosphere.*

Conditions at and above the surface of the soil, *i. e.*, in the ground and field strata.

(*a*) *Temperature.*—The above data on the temperature of the surface of the soil may be taken to represent essentially the temperature at the surface as well. There are no records of the temperature at various heights above the ground. Noticeable differences within the height of the trees present, are to be expected particularly in the cottonwood and other early stages where much bare sand is exposed.

(*b*) *Light.*—Animals are either positive or negative to the actinic rays of the spectrum (Congdon, '08, Mast, '11). Considerable work has been done by plant ecologists, on the measurement of light with photographic papers but its bearing on plant problems is questioned by some because the nonactinic portion of the spectrum is most important in the process of photosynthesis. It appears that these measurements are of much greater significance for animals than for plants. Zon and Graves ('11) have brought together the literature and discussed the methods of study (see especially several papers by Wiesner).

The light in the cottonwood stage is more intense than in any other of the habitats that we are to consider. Tests of the light in the beech woods and in the road adjoining, made with a Wynne exposure meter, show the following differences:

Location of Meter.	Time Required to Match Standard Tint.
Beech woods—darkest shadows	1,200 seconds.
Beech woods—medium shadows	180 "
Beech woods—brightest spots	10 "
Road on the north side of woods	3 "

While the above table shows a measurement of the actinic rays only, it indicates that in the beech forest, such rays at least, are diminished in intensity to from 1/3 to 1/400 that of full sunlight. On account of the great amount of reflection from sand, the light in the cottonwood stage is probably double that in the wagon road which is bounded on the south by beech woods and on the north by second growth timber.

(*c*) *Combinations or Complexes of Factors.*—As we have already pointed out (Shelford, '11), the animal environment is a combination of moisture, temperature, light pressure, materials for abode and food, all of which factors taken together constitute a complex of interdependences. These various factors are so dependent upon one another that any change in one usually affects several others. This property of environmental complexes is what makes ecology one of the most complex of sciences, and experimentation in which the environment is kept normal except for one factor, an ideal rarely realized in practice, even under the best conditions.

The efforts of ecologists, geographers, and climatologists have long been directed toward the finding of a method, of measuring the environment, which shall include a number of the most important environmental factors. De Candolle undertook to base the efficiency of a climate, for supporting plants, upon the mean daily temperatures above 6° C., this temperature being taken as the starting point of plant activity. Merriam has followed this lead and calculated total temperatures for many places in North America and made maps and zones based upon such totals. This system however, has been rejected by botanists and plant ecologists on account of much evidence both experimental and observational, which is quite out of accord with this view. The scheme has not been generally accepted by zoölogists outside of the United States Biological Survey. There is practically no evidence of an experimental sort, for the application of such a scheme to animals. Relative humidity has been suggested as an important index (Walker, '03) but does not properly express the influence of atmospheric humidity upon the animal body (Hann, '03, p. 53). The saturation deficit has also been suggested but does not take temperature into account.

1. *Evaporation.*

"The total effect of air temperature, pressure, relative humidity, and average wind velocity upon a free water surface in the shade or in the sun, is expressed by the amount of water evaporated" (Hann, p. 72). Since temperature in the season without frost is directly due to the sun's rays, light is in part included. In our latitude, clouds in summer slightly decrease the air temperature (Hann, p. 72). In winter however the temperature of cloudy days is higher. The strongest light is usually associated with the greatest evaporation. Yapp ('09) found that the rate of evaporation was directly correlated with temperature and illumination, but most closely correlated with relative humidity. From the standpoint of including many factors, the evaporating power of the air is by far the most inclusive and is therefore by far the best index of physical conditions surrounding animals wholly or partly exposed to the atmosphere. It is not however to be expected that it will hold good for all the factors under all climatic conditions, and for this reason, records of light, temperature, pressure, carbon dioxid, etc., should be made.

(*a*) *Effect of Evaporation upon Animals.*—In the case of man some observations have been made. According to Pettenkofer and Voit (fide Hann), an adult man eliminates 900 grams of water from his skin and lungs daily. Of this amount 60 per cent. or 540 grams come from the skin alone and changes in relative humidity of only 1 per cent. cause perceptible changes in the amount of evaporation from the skin. If evaporation from the skin and lungs is diminished, the amount of urine is increased, as in many cases are also the secretions of the intestines. Sudden changes in humidity make themselves felt in sudden increased or decreased blood pressure. The less dilute blood of dry climates operates as a stimulant and increases the functions of the nervous system. The consequences are excitement and sleeplessness (Hann, pp. 56–57).

Little has been done on the physiological effect of evaporation or desiccation upon animals. Various writers have found a loss of water associated with hibernation. Greeley obtained the same results with desiccation as with freezing (Greeley, '01; Bachmetjew, '99; Semper, '79, pp. 182–188). The reactions of animals to an atmospheric humidity gradient has probably never been studied. The chief conclusion to be drawn from the literature is that a high rate of evaporation is advantageous to some animals and decidedly detrimental to others. Attempts to keep insects and spiders which live exposed on the prairie vegetation, near Chicago, in the laboratory in screen cages containing vegetation, usually result in the death of the animals within a few hours. On the other hand, the same species will live in glass jars covered or partially covered with glass plates, long after the vegetation which was placed in with them has turned brown and has soured so that it gives off a bad odor. Special investigation would be necessary to determine the cause of this difference in the death rate, yet difference in the rate of evaporation from the animals' bodies is probably an important factor. After long and careful experimental studies dating far back into the history of plant physiology, plant ecologists have come to the conclusion, that the evaporating power of the air is the most satisfactory index of plant environments.

(*d*) *Evaporation in Forest Animal Habitats.*—Fortunately this has been investigated (Fuller, '11) in the five types of stations, viz., cottonwood, pine, black oak, oak-hickory, and beech. Fuller's first three stations were a little more mesophytic than ours. The data were obtained by using a porous cup atmometer. Evaporation from the atmometer is more nearly like that from an organism than is evaporation from any other device; it was devised by Livingston ('06, '08, '10, '10). It consists of a hollow cup of porous clay 12.5 cm. high, with an internal diameter of 2.5 cm. and a thickness of wall of about 3 mm. It is filled with pure water and connected by means of glass tubing to a reservoir usually consisting of a wide-mouthed glass bottle of one half liter capacity. The water, passing through the porous walls, evaporates from the surface, the loss being constantly replaced from the supply within the reservoir. Readings are made by refilling the reservoir from a graduated burette to a certain mark scratched upon its neck. For convenience in handling a portion of the base of the cup is coated with some impervious substance and before being used in the field, the instrument is standardized by comparing its loss of water with that from a free water surface of 45 sq. cm. exposed under uniform conditions. As a further check against error this standardization is repeated at intervals of six to eight weeks throughout the season (Fuller, '11). In Fuller's work, the bottles were sunk so that the evaporating surface of the instrument was 20–25 cm. above the surface of the soil.

Figure 2 shows the results of a season's study by Fuller. "The graph for the pine dunes is decidedly lower and more regular in its contour than that of the association which it succeeds. Its four nearly equal maxima would indicate that within its limits there was throughout the summer season a continuous stress rather than a series of violent extremes. On the whole it shows a water demand of little more than half of that occurring in the cottonwood dunes. Its greatest divergence is plainly due to the evergreen character of its vegetation and is seen on its low range in May and the first part of June, and again

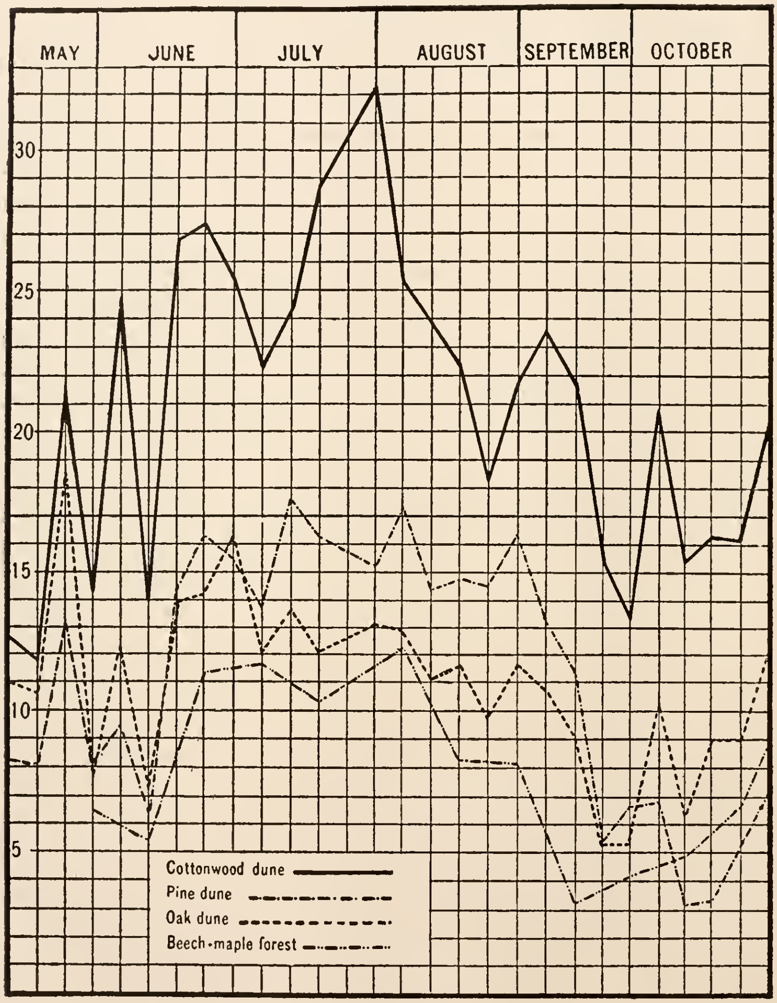

Fig. 2. Mean daily evaporation rates (cc. per day) in the ground stratum of four of the animal communities. (Courtesy of G. D. Fuller and the *Botanical Gazette.*)

in October when it falls below that of the oak dunes and is even less than that of the beech maple forest. This would give good reasons for expecting to find within this association truly mesophytic plants whose activities are limited to the early spring.

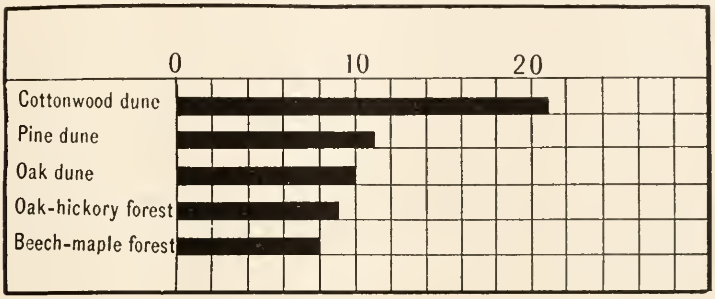

FIG. 3. Showing the comparative evaporation rates (cc. per day) in the ground stratum of the different animal communities from May to October. (Courtesy of Mr. G. D. Fuller.)

Evaporation in the various associations varies directly with the order of their occurrence in the succession (Figs. 3, 4). The differences in the rate of evaporation in the various plant associations studied are sufficient to indicate that the atmospheric conditions are most efficient factors in causing succession." (Fuller, '11.)

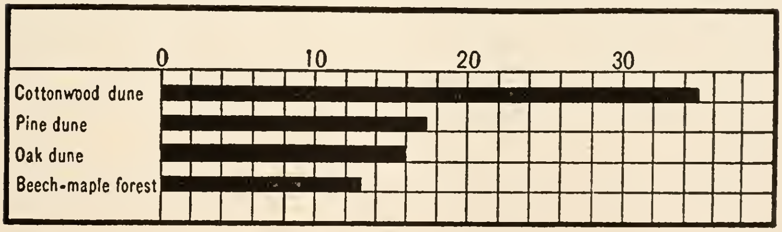

FIG. 4. Showing the comparative evaporative rates (cc. per day) in four of the animal communities on the basis of the maximum amount per day for any week from May to Oct. (Courtesy of Mr. G. D. Fuller and the *Botanical Gazette.*)

A comparison of Fuller's data with the tables and lists of animals shows that the distribution and succession of animals is *clearly correlated* with the *evaporating power of the air.* Further comparison with the description of stations (p. 61) shows that the evaporating power of the air may be taken, in this case, as an index of the materials for abode, etc.

4. *Influence of Physiography and Vegetation upon Animal Habitats.*

In some cases the evaporating power of the air is apparently largely controlled by the vegetation and in others largely by physiographic conditions while as a rule both physiographic conditions and vegetation play important rôles. The importance of the combined effect of physiographic conditions and vegetation is well shown on the steep clay bluffs of Lake Michigan. For example, at Glencoe, Ill., erosion has rendered the bluff steep and brought the ground water near the surface in some places (Shelford, 11[4]). Forest animals occur among the shrubs and under the dead sweet clover (Fig. 5).

Table IV.

Showing forest animals in the early stages of forest development of a clay bluff of Lake Michigan. Subterranean and ground strata, 1; bare clay, 2; sweet clover, 3; shrubs, goldenrod, etc., 4; sapling stage; animals same as in 5, the oak-hickory forest.

Common Names.	Scientific Names.	1	2	3	4	5
Tube weaver	*Aglena nævia* Wal.	×	×			
Lycosid	*Pardosa lapidicina* Em.	×	×			
Carolina locust	*Dissostiera carolina* Linn.	×	×			
Mud dauber	*Pelopæus cementarius* Dru.	×	×			
Tiger-beetle larvæ	*Cicindela purpurea limbalis* Klg.	×	×			
Sow bugs	*Porcellio rathkei* Brandt		*F*	*C*	*A*	*A*
Centipede	*Geophilus* sp.		×	×	×	×
Snail	*Polygyra thyroides* Say		×	×	×	×
Snail	*Pyramidula alternata* Say		×	×	×	×
Snail	*Polygyra monodon* Rach			×	×	×
Tiger-beetle larvæ	*Cicindela sexguttata* Fabr.			×	×	×
Snail	*Polygyra albolabris* Say			×	×	×
Slug	*Phylomycus carolinensis* Bosc.			×	×	×
Yellow-margined millipede.	*Fontaria corrugate* Wood			×	×	×
Centipede	*Lyasopetalum lactarium* Say			×	×	×

All of the species beginning with *Geophilus* are commonly found in the oak-hickory forest. On the covered bluff however, where the moisture content of the soil is great and the dense sweet clover and the shrubs make a good covering we find these animals associated with the earliest stages of vegetation development. Shade and moisture here appear to be the determining factors. We note here then that the forest floor conditions are in advance of the forest while on the dry well-drained sand they lag behind in succession.

Some investigators have questioned the importance of vegetation to animals and we note here that the distributions of plant and animal species are not always correlated. If one

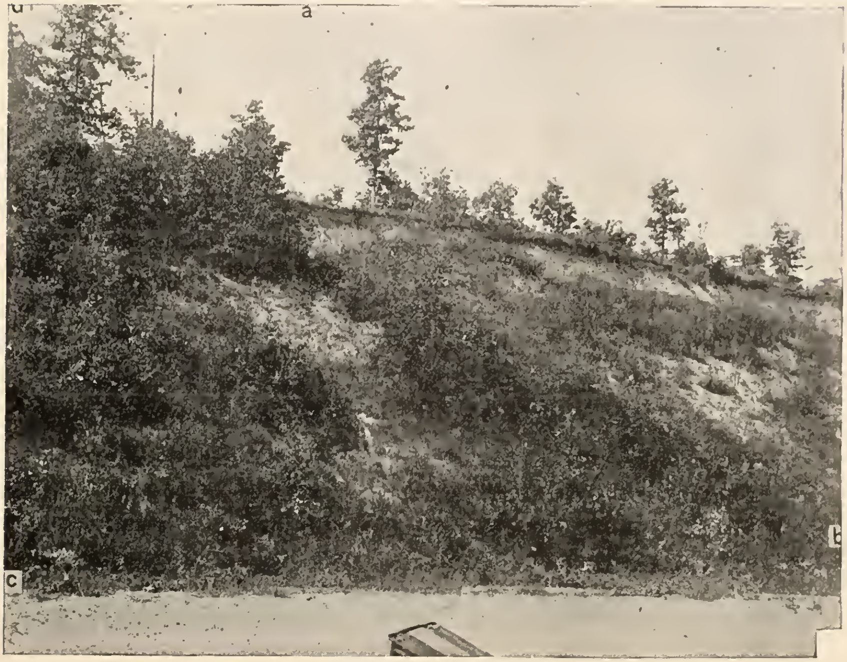

FIG. 5. The bluff of Lake Michigan at Glencoe, Ill., showing several stages of forest development. To the right of an imaginary line *a–b* are small areas of the habitats shown in Table IV., in columns 1 and 2. Within the triangle *a–b–c* are areas of the same habitat invaded by shrubs under which are found forest animals. To the left of *a–c* is an area of shrubs and saplings which has a full quota of forest floor animals. (Reprinted from the *Journal of Morphology*.)

refers to *species* of *plants* and *species* of *animals* then the vegetation very often is not correlated with the distribution of the animals. If on the other hand one means that the plants are controllers of physical conditions, then vegetation can be said to be of very great importance.

5. *Stratification of Conditions.*

An inspection of the tables and the discussion following them shows that different animals which do not burrow into the ground inhabit different levels of the forest. For example *Acrosoma* *spinea* Hentz builds its web 1–3 ft. above the ground while *Acrosoma gracile* Wal. builds 4–6 ft. above the ground (see Dahl, '08).

Table V.

Evaporation from Porous Cup Evaporimeters in Different Strata of a Summer Dry Marsh, Cambridgeshire, England, During Three Periods Between July 9 and September 8, 1907.
(Yapp, '09, p. 299 and 294.)

Year.	Height above Ground.	Ratio of Evapor.	Temperature.			
			Mean Max.	Mean Min.	Mean.	
1907	5 ft. 6 in. to 4 ft. 6 in.	100.00	22.1	6.6	16.5	Well above vegetation.
1907	2 ft. 2 in.	32.8	23.0	—	—	A little above the mid height.
1907	.5 in.	6.6	18.0	7.1	14.1	
1907	soil	——	12.7	11.2	11.8	

The above table shows marked differences in the rate of evaporation, considerable differences in temperature at the different levels and both due largely to vegetation. Differences in light are also to be expected. Sherff ('12, p. 420) has found conditions similar to the above by a two months' study of evaporation on Skokie marsh near Chicago. The evaporation there was three times as great at a height of 1.95 m. as at the surface of the soil in among the plants of Phragmites. Mr. Harvey has also secured similar (unpublished) results on the prairie at Chicago Lawn, Chicago.

It has been long recognized that there are distinct growth-form strata in nearly all plant formations, pelagic algæ formations being a possible exception. The data of Sherff and Yapp indicate differences in conditions in the strata of grass formations and associations. Greater differences are to be expected in the different strata of forests and shrub covered areas. Mr. Fuller informs us that there are marked differences in the structure of leaves at different levels of the same forest tree.

6. *Apparent Anomalous Distribution.*

Are physical conditions sometimes similar when vegetation and landscape aspect are very different? That they are is clearly suggested when we compare the forest and the shrub covered bluff where forest animals occur. Plants grow from seeds only under a very limited range of conditions. However if trees are given a few years' growth under favorable conditions they will be successful under a great range of conditions. The great age to which trees often live and the slowness with which they grow makes it possible for conditions to change while the trees still live on with changes only in leaf structure. It is to be expected that the distribution of animals is correlated with the occurrence of seedlings or of quick growing plants or at least with leaf structure types rather than strictly with species of trees. These facts suggest that there are two types of cases in which physical conditions and forest conditions are not in accord. In the first case atmospheric conditions become favorable for forest animals before any woody plants have been able to grow, in the second, woody plants remain after conditions have become unfavorable for forest animals; both are due to lagging behind of vegetation; both are very local and of minor significance.

A comparison of the data of Yapp (Table V.) and Transeau (Fig. 6) shows a difference between the evaporation of the lower

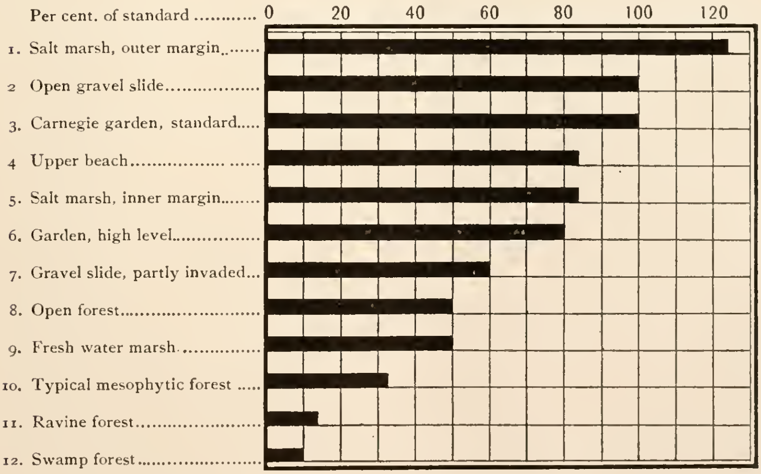

FIG. 6. Showing the comparative evaporation rates (c.c. per day) in the ground stratum of several animal habitats on Long Island during July and August. (After Transeau, courtesy of the *Botanical Gazette.*)

stratum of a marsh and the free atmosphere above, comparable to that found by Transeau between the lowest stratum of the mesophytic forest and the open gravel slide. An inspection of Transeau's data (Fig. 6) on evaporation indicates several obvious cases of similarity; *e. g.*, we note that the rate of evaporation is about the same in the fresh water marsh and the open forest. The data at present available do not justify definite conclusions, yet it may be suggested that there are various stations in strata of the different plant communities where the conditions of the physical factors are essentially identical, but where the necessary materials of abode, especially those used in breeding, are sometimes wanting. Their absence alone is sufficient to prevent animals of specialized habits and structures from taking possession of situations otherwise entirely suitable.

The reasons for the wide distribution of some animals in the forest stages which we are considering are no doubt various. For example *Zonites arborens* (Table I, p. 67) is rare in the early stages and is confined to the lower and moister localities. If *Epeira domicilorum* is a species of stable physiological makeup we can offer no explanation for its peculiar distribution (Table II, p. 69). A species may have its critical period in the early spring when the leaves are off the trees and the condition of the atmosphere similar in all (see Fig. 2) stages or may live at higher levels in the denser and older stages, and thus be surrounded by similar atmospheric conditions, but we are not warranted in assuming either of these causes here.

Another striking feature of the distribution of many beetles, bugs and spiders, and Orthoptera is the fact that they are found in open woods, edges of woods, on the vegetation of marshes and over the water of small ponds in which vegetation is growing. In this way many species are found to occur in what at first appear to be very unlike situations. *Lygus pratensis*, *Tripleps insiduosus*, and *Euschistus variolarius* which occur on the vegetation of the margins of swamps, of the black oak forest dunes and on prairies and agricultural lands may serve as examples. Shull ('11) has pointed out similar facts as one of the difficulties in the way of ecological classification of Orthoptera and Thysanoptera. Such species as the bugs mentioned above are said to occur "everywhere," although they are rarely found in moist woods or in any situation in which they are not fully exposed to the sun and may always live in similar conditions.

There are great differences between open prairies and closed forests. Shimek ('11) found that the evaporation in the undisturbed groves in eastern Iowa during July and August was very much less than that in the prairies adjoining. From the free surfaces of pans set in the ground so that the water which they contained was level with the surface of the soil, the evaporation of the groves was about 27 per cent. of that of the prairie; with cup evaporimeters about 37 per cent. and with Piche evaporimeters about 47 per cent. This is about the same as the difference on Long Island between the inner side of Transeau's salt marsh dominated by grass-like plants and his mesophytic forest. Sherff ('12) found the evaporation in a marsh forest to be a little less than that in the beech maple and from 1.8 to 2.6 times as great as in the lowest stratum of a marsh. While differences and similarities of physical conditions are sufficient to account for many peculiarities of ecological distribution, it must be recognized that the *same species may occur under different conditions and show difference in mores* (Bohn, '09, Allee, '12).

5. *Agreement of Plant and Animal Communities.*

Before discussing the problem of agreement between plant and animal communities, it is necessary to state what is meant by agreement. According to present developments of the science of ecology *plant and animal communities may be said to be in full agreement when the growth form of each stratum of the plant community is correlated with the conditions selected by the animals of that stratum.* Questions of agreement are primarily questions for experimental solution. Two types of disagreement are to be expected. We may illustrate the first by a bog or marsh community. Considering plants rooted in the soil we note that water is secured from the soil by the roots and is lost through the leaves and twigs. Accordingly since bog soil is unfavorable, due to the presence of toxins or to other causes, plants growing in it do not secure water easily even when the quantity of soil water is great. *Such plants have xerophytic structures (which tend to check the loss of water) developed far beyond the requirements of the atmospheric conditions surrounding their vegetative parts.* It is improbable that the animals inhabiting a bog-vegetation field-stratum would *select* atmospheric conditions such as produce equally xerophytic structures under *favorable soil* conditions. We may therefore expect disagreement. The smaller plants such as fungi, algæ, etc., are related to the strata of soil and atmosphere exactly as the smaller animals and as *much disagreement* is to be expected between such plants and the rooted vegetation as between the rooted vegetation and animals. It must also be noted that the xerophytic structures of the plants of *unfavorable* soils may have important influence upon ectophytic plants and animals and in part counteract the effect of favorable atmospheric conditions.

The second type of disagreement is represented by cases in which the vegetation is said to lag behind. We have noted that on the clay bluff, conditions become favorable for inconspicuous plants and forest animals as soon as the growth of the pioneer vegetation gives shade to the soil. In other cases woody vegetation remains in situations where the conditions have become unfavorable for it and the less conspicuous plants and some of the animals have disappeared. We may expect lack of accord within and between plant and animal communities under such conditions. In these cases, however, conditions are only *temporarily out of adjustment*, due to rapid physiographic changes and we note from the data presented that plant and animal communities are usually in agreement. The exceptions are often apparent only and due to the emphasis of *species* instead of *mores* and *growth form*.

VI. General Discussion.

At this point we may note certain aspects of the basis for the organization of ecology into a science. It is possible to characterize the communities of the forest in physiological terms though we cannot be as definite as is to be desired, until *mores* have been studied in detail. Taking the communities one by one and stratum by stratum we may note obvious characters.

A. Pioneer Communities.

The communities of the cottonwood, pine and black oak stages may be designated as pioneer because of the presence of bare mineral soil.

1. *Subterranean and Ground Strata.*—(*a*) The cottonwood community is characterized by animals which breed and spend the dark and cloudy days chiefly below the surface of the sand. They are very largely diurnal and predatory, are exceedingly swift and wary. The burrowing spider (*Geolycosa pikei*) is one of the few nocturnal animals.

(*b*) The pine community is characterized by similar *mores*, but is to be distinguished from the preceding by the presence of many animals which prefer sand that is less shifting and which is slightly *darkened by humus* (Shelford, '07). Animals requiring "cover," such as the lizard, the blue racer, a few ground squirrels, etc., give character because of their absence from earlier and later communities.

(*c*) The black oak community represents the climax of diversity of the subterranean and ground strata. The bare sand *mores* continue in the open spaces, which we have designated as transition areas. Leaf cutters are now present while among the burrowers, the root borers (Prionids and Lucanids) work on the roots of the decaying trees. The behavior differences between this and the preceding communities are differences of detail which, for the making of deductions, would require much careful study.

2. *Field and Shrub Strata.*—The field and shrub strata of the cottonwood, pine and oak communities are less easily characterized. The cottonwoods of the beach are far less commonly infested with aphid galls than are trees of the same species growing in less exposed situations. Furthermore we have never found any of the lepidopterous larvæ such as *Basilarchia archippus* near the beach. Animals living exposed upon the trees are few in number. The same general conditions obtain on and among the pines but spiders are more numerous. On the black oak the number of phytophaga is increased and the number of galls appears to be greater than in the later stages; the inhabitants of the herbaceous vegetation are chiefly those found in

open situations such as prairies and roadsides, where the physical conditions are similar. Some animals of the same species which make up the black oak community were taken from a roadside and after being mixed with the inhabitants of the shrubs of the beech forest, were placed in a light gradient. Soon the insects and spiders of the two communities separated sharply from each other, the beech-inhabiting species going to the darkest end while the roadside species all crowded to the light.

B. Later Communities.

With the coming in of red oak true forest with the mineral soil largely covered with humus and leaves is present and very different *mores* obtain. The diurnal diggers are practically absent. Snails, beetles, grasshoppers, spiders and myriopods living under bark, decaying wood, and leaves, avoiding strong light and requiring moisture, are the chief types. The *mores* are typically forest in character. The differences between these and the later stages are those of detail and degree which need careful study. In general with a lessening in the severity of the conditions, there is a proportional increase in the use of the vegetation as a place of abode.

In the field and shrub strata, we note that the animals of the cottonwood, pine and oak stages are characteristic of open dry situations, requiring or tolerating strong light, while those animals of the red oak, hickory and beech stage are negatively phototactic to light of the same intensity, as shown by mixing the animals in a gradient.

The animals of the tree stratum are few and scattered in the cottonwood, pine and black oak stage while animals enclosed in galls or cases are common if not dominant. In the red oak, hickory and beech stage phytophaga are often gregarious and numerous. The vegetation is used more and more for a breeding place as the forest increases in denseness. Groups such as orthoptera, beetles, bees and wasps, are represented more and more by species which make use of the vegetation as forest development goes on. The tree strata of all the forest stages are characterized by species given to frequenting a limited number of kinds of trees.

C. General Considerations.

We note that the distribution of animal species which occur chiefly on a particular plant species or on closely related species of a group, do *not often occur everywhere* that the plant or plants occur, and if they do there is a marked difference in the number of individuals. Such phenomena appear to be matters of common observation among naturalists. While they are still subjects for investigation, there is much evidence that the local distribution of the phytophaga is not that of the food plant or plants but is limited to a certain portion of the local range of the plant, by differences in the physical conditions, or the growth form of the plant or both. The food plants of phytophaga having a number of food plants are usually those growing in associations. The fauna of trees growing in different communities or under different conditions are probably *commonly different.* The differences in the *mores* of the communities outlined above are clearly correlated with factors known to be of importance in the behavior and physiology of animals. These are materials for abode, soil moisture, light and the condition of the atmosphere.

The more important features of the environment of an animal are selected through its reactions, which are probably innate or instinctive (Wheeler, '10, p. 159; Shelford, '11^4, pp. 556–582; Hancock, '11, p. 327; Herrick, '05, p. 201). Different species usually select different habitats or different strata in the same habitat. It is well known among naturalists and experimenters that *different species* usually have *different mores* (Brehm, '96, p. 73).

Animals of the same species show behavior differences in different habitats (Jennings, '06, Ch. XXI.; Shelford, '11^4, p. 584; Allee, '12). Bohn found, that the sea anemones living near the surface of the sea where the wave and tide action are strongest, showed more marked rhythms of behavior in relation to tide than those living lower down where the action of the tide and waves is less marked (Bohn, '10, p. 156; Holmes, '11, p. 155). These rhythms disappeared slowly when the animals were removed from the tide to the aquarium. Many such cases are probably to be found in the natural history literature. For example the chipmunk differs in behavior under different conditions (Wood, '11, p. 523). Abbot ('70, p. 104) makes a similar statement about fish. It is apparent then that one species may have *several mores* (Bohn, '10 et al.). Different species may sometimes have *identical mores;* these cases are usually separated geographically (Shelford, '11, p. 32; '11[2], p. 147; '11[4], p. 604). In addition to these relations, the relation of ecology to species is largely a matter of language, names being necessary as a means of referring to animals.

Animal ecology has very much in common with plant ecology. Diatoms, flatworms and many other marine animals and plants meet the same conditions in the same or similar ways (Loeb, '06, p. 121; Bohn, '10, p. 156; Holmes, '11, p. 155). Sessile animals, such as reef-forming corals, show growth form differences (Woods-Jones, '10) under different conditions, just as sessile plants do. Comparable plants and animals show comparable responses. The physiological life history aspect of plant ecology (Ganong, '07) is parallel with the same phenomenon in animals, but the activities of motile animals correspond roughly to the growth-form phenomena in sessile plants (Shelford, '11[4], p. 593). Results of study of the environment are equally applicable to plants and to animals. Since *mores* and *growth-form* are correlated with the *environment* much progress can be made by the *study* of the environment; in fact, study of the environment is necessary for progress.

On the other hand the study of the environment must be accompanied by experiments designed to determine the relative importance of the different factor *to animals,* or the results, like so many of our meteorological records, will prove to be of questionable value for the purpose for which they are intended. In the case of the forest animal communities which we have studied, experiments must be undertaken to determine the physiological relations of animals to materials for abode, soil moisture, light and the condition of the atmosphere before the subject can progress beyond the suggestive stage which this paper necessarily represents.

Ecology or ethology of single isolated species is a very old branch of biological study. The developments of the last twenty years have been in the direction of organization of these isolated facts into a science on the basis of *mores*, including *habitat preferences.* The similarities between the response phenomena of plants and animals have led in the direction of the organization of a branch of biological science embracing both plants and animals. It is this organization, or the possibility of organization, which we are attempting to introduce here. The experimental work cited above is adequate to indicate the lines along which further investigation should be directed and that *the mores problem, which includes the habitat preference problem, is the central problem of ecology.*

VII. Summary.

1. The development of forest on sand or other mineral soil is accompanied by an almost complete change of animal *species* and probably by a complete change of animal *mores* (pp. 67–72).

2. Forest development is accompanied by marked changes in soil and physical factors; animal distribution is more closely correlated with differences in *physical factors* than with species of plants (pp. 73–82).

3. For animals living in the soil, the moisture equivalent, or the wilting coefficient for a standard plant, is the best index of the moisture available to the animals (p. 74).

4. The rate of evaporation or the evaporating power of the air is probably the best index of the conditions of the atmosphere (p. 77).

5. The rate of evaporation, temperature, etc., have been found to be very different in the different communities and also different in the different strata of the same communities. The amount of evaporation in animal communities is directly related to their order of occurrence in succession (p. 81).

6. Plant and animal communities are divisible into strata which represent vertical differences in physical conditions. The bodies of many plants occupy several strata but their vegetative parts are usually in some particular stratum. Land animals are comparable to smaller non-rooted plants such as algæ, lichens and fungi. Many animals carry on different activities in different strata, but are to be classed primarily with the stratum in which they breed (p. 84).

7. Succession of all the animals of the forest communities under consideration is comparable in principle to that in ponds. Succession is due to an increment of changes in conditions produced by the plants and animals living at a given point. Animals through their effect upon the soil play an important though minor part in the process (pp. 73, 75).

8. The various animal species are arranged in these communities in an orderly fashion and the *dominating animal mores* are correlated with the *dominating conditions* (pp. 81, 89–90).

9. Taxonomic (structural) species usually have distinct *mores*, though the same species often has different *mores* under different conditions, and different species may have the same *mores*. *Species* and *mores* are therefore not synonymous (pp. 91–92).

10. Ecology considers together *mores* that are alike or similar in their larger characters (p. 92).

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THE OVUM OF THE NINE-BANDED ARMADILLO. GROWTH OF THE OVOCYTES, MATURATION AND FERTILIZATION.

H. H. NEWMAN.

TABLE OF CONTENTS.

I. Introduction 100
II. Literature on mammalian ovogenesis 103
III. Material and method 104
IV. Scope and outline of observations 106
V. Development of the Graafian follicles 107
VI. Development of the ovocytes with especial reference to vitellogenesis and to the comparative rate of growth of nucleus and cytoplasm . . 112
VII. Nuclear changes during the growth period of the ovocytes 117
VIII. The first polar spindle 120
IX. The first polar body and the second polar spindle 122
X. The second polar body and the female pronucleus 124
XI. An anomalous case of a third polar spindle 124
XII. Fertilization 125
Summary and conclusions 126
Literature list 128

I. Introduction.

Interest in the armadillo ovum is due chiefly to its unique capacity for polyembryonic development. As has been shown (Newman and Patterson, '10) the ovum develops throughout the early stages of the embryonic period as a single blastodermic vesicle and, only after the differentiation of the primary germ layers, divides visibly into four embryonic primordia. That the four embryos of a litter are always of the same sex has also aroused the interest of biologists, especially those engaged in researches on the problem of sex determination. Since in the armadillo sex would seem to be predetermined in the undivided oosperm an examination of the cytology of both male and female sex cells should be made.

Many conjectures as to the underlying cause of polyembryony in the armadillo have no doubt been made by all who have taken an interest in the phenomenon. Among those that have been most commonly suggested are the following:

1. On the basis of a cytological examination of one pair of ovaries Rosner ('01) concluded that four adjacent follicles fuse in such a way that four eggs are thrown into a single follicular cavity; on the rupture of this compound follicle four eggs are discharged simultaneously, descend the fallopian tube held together in a mass by means of their discus proligerus cells, become fertilized, undergo cleavage and come to a common point of attachment in the uterus; subsequently the contiguous walls of the four blastocysts atrophy and a single vesicular chorion is produced. According to Rosner then polyembryony does not exist, but merely the appearance of polyembryony, due to an early fusion of four blastodermic vesicles.

2. It has been suggested that the ovum might give off two large polar bodies and that the first of these might divide, thus producing four potential ova within one zona. These would be separately fertilized and would produce a morula apparently simple but actually quadruple. Subsequently the four embryonic components would segregate themselves and produce the quadruplets. This view also denies the reality of polyembryony.

3. There might occur an early fusion of four ovogonia or ovocytes to form a tetra-nuclear germ cell, which would be fertilized by as many spermatozoa as there were female pronuclei and thus give rise to a quadruple embryonic vesicle.

4. The two maturations might occur within the cytoplasm of the egg, without the extrusion of polar bodies. We would have in this case an egg with four pronuclei which, when fertilized with four spermatozoa, would be able, conceivably, to produce four embryos.

5. There might occur two successive parthenogenetic divisions of the female pronucleus, prior to fertilization, which would require four spermatozoa for their fertilization and would thus account for the observed conditions.

6. The cytoplasmic materials of the ovum might be physiologically isolated into quarters during some period of the ovarian history. Such a condition might conceivably foreshadow the actual isolation of embryonic primordia as it occurs at a fairly early period of embryonic development.

7. The cause of specific polyembryony may lie in factors

strictly external to ovum, among which one of the most probable is in some way associated with the bilaterality of the uterus. A discussion of this possibility would be foreign to the topic in hand and must be postponed for subsequent treatment.

The first five possible explanations will probably appear to the reader to be without foundation and far-fetched. Every one, however, has been offered by serious-minded biologists. The majority of these explanations have already been shown to be untenable, some require further refutation. It is one of the purposes of this paper to remove the latter from further consideration. There is a small amount of evidence in favor of the sixth suggestion, but it is far from convincing. So it would appear that the stimulus to specific polyembryony must be looked for in some external factors, the character of which we are not prepared to discuss at present. This being the case a study of the history of the female germ cells can furnish only negative evidence on the main question at issue and would therefore lack the inherent interest that usually attaches to positive results, were it not that a considerable number of interesting, and, I believe, important facts, apparently quite unrelated to the phenomenon of polyembryony, have come to light. These facts are therefore presented partly to pave the way for further cytological studies but principally because they appear to possess a value quite independent of any of the general problems so far suggested by studies of the armadillo.

It is shown in this paper that the armadillo ovum bears a remarkably close resemblance to that of *Dasyurus*, the native marsupial cat of Tasmania, described by Hill ('10). In both *Dasyurus* and *Tatu* the ovum, at maturity, exhibits an inverted "telolecithal" condition. The genesis of this peculiar state of affairs is traced through the growth period of the ovocyte and incidentally a description of the parallel development of follicle, ovocyte and germinal vesicle during this extensive period is presented in the belief that this phase of ovogenesis has been too largely neglected by students of the maturation and fertilization of the mammalian ovum. It will be noted also that the armadillo ovocyte is especially favorable for the study of chromosomal behavior during the maturation processes and that it is possible with some assurance to enumerate the elements of the chromosome complex. This should furnish a useful companion study to that of the spermatogenesis which is being worked out by Dr. J. T. Patterson. Finally, the facts here presented serve to banish any hesitation that may at any time have been entertained as to the validity of the assumptions upon which are based the studies of the predeterminative and epigenetic factors concerned in the development of the definitive characters of the armadillo quadruplets, studies which were outlined in a former publication (Newman and Patterson, '11) and which are at present being carried on with a much more adequate collection of material.

II. Literature on Mammalian Ovogenesis.

Considered solely as a contribution to our knowledge of the maturation and fertilization processes of mammalian ova the present study would be well worth presentation owing to the fact that the work in this field has been confined to three orders of mammals, Rodentia, Cheiroptera and Carnivora. Nothing is known of the conditions in any Edentate. It is a pleasure then to add to this brief list not only an additional order but one in which the ovum is of a type more primitive than any previously noted for *Eutheria*.

Our knowledge of ovogenesis in the Cheiroptera is limited to one species, *Vesperugo noctula*, described by O. Van der Stricht in 1909. The only representative of the Carnivora which has received adequate attention is the domestic cat, the maturation and fertilization of which have been recently worked out in detail by Longley ('11). The rodents however, have furnished the basis for numerous elaborate studies. Conditions in the guinea-pig, the rat and the mouse are known in detail and especially is this the case with the mouse, upon which no less than eight investigations have been published. All of this rather voluminous literature has been recently reviewed by several authors and for details the reader is referred to the papers of Kirkham ('07), Sobotta and Burckard ('10), Long and Mark ('11) and Longley ('11).

We have then adequate accounts of the ovogenesis of only five species of mammals: the bat, the cat, the guinea-pig, the rat and the mouse. Of these all but the bat are domesticated forms; so the armadillo is the second species of wild mammal whose ovogenesis has been investigated.

Longley ('11) points out very pertinently that the failure on the part of investigators to secure material for the study of ovogenesis in the higher mammals is due partly to the difficulty of procuring the eggs of these forms in the conditions needed and partly to the fact that the ovaries of large animals are too bulky for convenient investigation, involving as they do a study of serial sections of a comparatively enormous mass of tissue.

III. MATERIAL AND METHOD.

In the pursuit of the study of the maturation and fertilization processes of wild mammals two courses are open to the investigator. He may breed them in captivity, a precarious and not often successful undertaking involving the killing of many animals tamed at great pains. The only remaining course of action is that which has been resorted to in the present investigation, namely, to rely upon the chance collection of favorable stages in the ovaries or fallopian tubes of freshly captured females during the period of œstus.

In the case of the armadillo of Texas very serious difficulties are encountered in keeping the animals and breeding them in captivity. In the first place they appear to breed but once a year and would therefore have to be kept in considerable numbers for a long time in order that an adequate collection of stages could be made. Experience has shown that the animals are extremely difficult to domesticate. They need much territory for the exercise of their normal functions and apparently would breed only if allowed to burrow in the ground as is their custom. In addition to these obstacles to rearing, the animals, as they have come under my observation, are almost invariably badly infested with flesh parasites that rapidly gain the ascendency if the animals are subjected to conditions somewhat less favorable than the normal.

In view of these conditions I have been forced to rely on serial sections of ovaries and the attached fallopian tubes for my studies of maturation and fertilization. The former process is, I am convinced, well illustrated in the material at hand; the latter has been found only in one case, but this has all the earmarks of a normal fertilization stage and is therefore accepted as typical for the species, pending further evidence which may or may not be forthcoming.

During three years material for this paper has been collected and studied. Ovaries of adult and young females have been fixed in various fluids and studied at all stages of the sexual cycle. As a rule the best stages have been obtained from the ovaries of large females taken at the height of the mating season. Ovaries of pregnant females show little of interest in this connection.

Out of a considerable variety of fixing agents used it soon became apparent that by far the most efficient for nearly every purpose was Zenker's fluid. Fleming's, Gilson's, Bouin's, Petrunkewitsch's and formalin Zenker gave uniformly less satisfactory results and were not used after the first few trials. For the study of vitellogenesis the ovaries were fixed in 10 per cent. neutral formalin and favorable free-hand sections were stained in Sudan III. This material, when counterstained with a weak solution of methyl green was also best for measurements of ovocytes, as there was practically no shrinkage.

A variety of staining processes gave satisfaction, but the best for both nuclear and cytoplasmic details proved to be Bensley's copper chrome hæmatoxylin process. This stain gives as sharp definition of chromosomes as does Haidenhain's iron hæmatoxylin method and in addition stains acromatic nuclear materials and cytoplasmic structures admirably. On account of the standard character of the iron hæmatoxylin technique, however, this stain was used throughout as a control. For certain special points several other staining combinations were employed, notably toluidin blue and acid fuchsin, neutral safranin and acid violet, and thyonin and erythrosin. These served to bring out certain differentiations that could be discovered only by their aid.

IV. Scope and Outline of Observations.

The present study begins with a consideration of conditions found in ovocytes at the beginning of the period of growth, just before they have acquired primordial follicles. It appears necessary to begin the study of maturation thus early partly because the prophases of maturation appear to be well under way at this period and partly because the development of the follicle and the relations of the ovocytes to the follicle are of fundamental interest in connection with the investigation into the causes of polyembryony. The growth of the ovocyte is accompanied by growth and modification of the follicle and the development of both culminates in a condition which would normally be followed by ovulation. Failure of ovulation, however, is the fate of the vast majority of developing ova, owing partly to their position in the ovary but chiefly to the influence of pregnancy, the occurrence of which inhibits further ovulation. These ova which have reached full size and are in every way mature and ready for ovulation, occasionally complete the process of maturation in a manner identical with that which normally occurs only in ova which have been fertilized, and under some conditions develop parthenogenetically through the cleavage period, as I have determined quite conclusively. In lieu of any data concerning the completion of the second maturation division in tube eggs, it is considered a legitimate procedure to substitute an account of the condition seen in these ovarian ova in which the maturation process has proceeded beyond the stage ordinarily seen in such ova. That this is a justifiable use of material is shown by the fact that in all species where both the normal process of maturation, and that seen under conditions identical with those just indicated, have been studied, there has been found no essential difference between them.

The study then may be conveniently divided into two parts, the first dealing with processes taking place in normal follicles up to a period when ovulation would normally take place, and the second with the completion of the maturation in follicles undergoing the early stages of follicular atresia. In all other species where the facts are known ovulation occurs during the second maturation division, after the first polar body has been extruded. Stages up to this period are fairly numerous in the present material, but there are only two examples of ovarian ova completing the second maturation. Without further preliminaries then it will be understood that all stages of the process as here described are normal with the exception of those described and shown in Figs. 42 and 43.

It seems best to describe first the stages of follicular growth, for it is very convenient to refer various stages of the developing ovocyte to certain figured stages of follicular differentiation. The growth period of the ovocyte, from the condition when it is without a follicle to the period of maturity, is next taken up, and incidentally the process of vitellogenesis and its consequences receives attention. For the sake of completeness and as a transition to the next study the matter of nuclear growth as compared with cell growth is considered. Finally the nuclear changes, principally those concerned with the formation of the chromosomes and the acromatic structures of the maturation spindles, are described in detail since chief interest appears to center upon these changes rather than upon any transformations or reorganization of the cytoplasm.

V. Development of the Graafian Follicle.

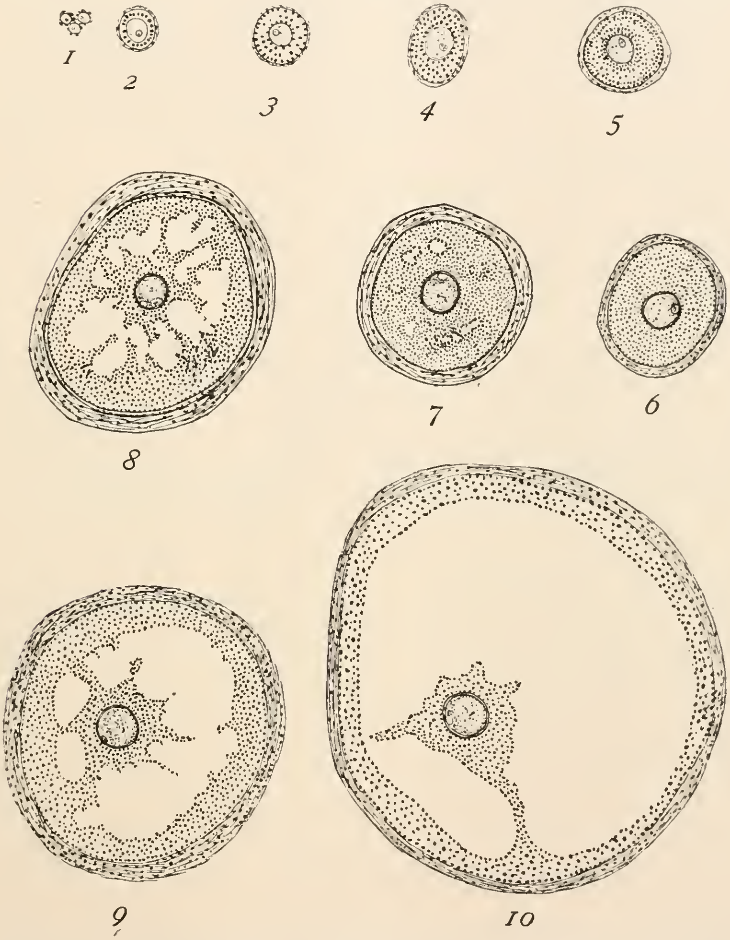
There appears to be nothing especially specific in the process of folliculogenesis as it occurs in the armadillo. Comparisons have been made stage for stage with that of the cat, the ovaries of which are of about the same size as those of the armadillo, and only very minor differences have been noted. This fact, in itself a matter of no particular moment, gains importance when it is remembered that Rosner ('01) attempted to explain away polyembryony on the basis of a very peculiar sort of fusion of adjacent follicles. The following account will serve finally to set at rest any misconceptions that may have been engendered by Rosner's unfortunate account. In addition we shall be afforded a sort of convenient time schedule upon which to hang the descriptions of the other processes with which the present study is concerned; for we shall be able to refer any particular phase of ovocytic or nuclear development to some definite stage of follicular growth. Full grown ovocytes, for example, are invariably to be found in follicles of stage 10 (Fig. 10), while mature or maturing ovocytes are found only in follicles of type 11 (Fig. 11). It is fortunate for the student of ovogenesis that the condition of the follicle furnishes such an accurate index of the more important ovocytic changes, since it enables him to search through a large amount of material with low powers of the microscope and to detect readily certain follicles which he may examine with the assurance that he will find the desired stages of ovocytic or nuclear development. Hence the following very brief account of folliculogenesis is offered largely for the purpose of rendering the subsequent ovocytic and nuclear history more easily followed by the reader.

Stage 1 (*Fig. 1*).—The primordial follicle is just in process of establishment. Frequently nests of young ovocytes are found to be surrounded by primary follicular cells, giving the impression of several ovocytes in a single follicle. This is not really a pluriovular follicle but simply a stage prior to the completion of the establishment of the true primordial follicle. It is, however, positively the only condition in the entire process of ovogenesis in which I have found anything that might be looked upon as a fusion of follicles. The diameter of the follicle at the time when the ovocyte is surrounded with follicle cells averages about .03 mm.

Stage 2 (*Fig. 2*).—The single layer of primary follicle cells takes the form of a simple cubical epithelium, sharply cut off from the capsule of stroma cells. Both ovocyte and follicle cells have grown considerably, for the average diameter of follicles of this stage is about .1 mm.

Stage 3 (*Fig. 3*).—The simple epithelium of the follicle becomes compound. The figure shows a three-layered condition. Follicles at this stage have an average diameter of about .15 mm.

Stage 4 (*Fig. 4*).—There is a strong tendency at this stage for the follicle to become elongated on account of the more rapid proliferation of the cells at the two ends. This condition is usually accompanied by an elongation of the ovocyte as shown in Fig. 15. This phenomenon is of such frequent occurrence that I am inclined to offer the tentative suggestion that a bilaterality of the ovocyte might be initiated here, which under certain conditions might produce a physiological isolation of the two halves of the germ cell and might account for the production



FIGS. 1 to 10 (inclusive) show ten stages in the development of the definitive follicle (×50).

of the paired embryonic primordia that are such noteworthy features of the early development as shown recently in Patterson's photographs.[1] Follicles at this stage measure on the average about .15 × .2 mm.

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[1] These photographs were exhibited at the Urbana meeting of the Central Branch of the American Society of Zoölogists, held in April 1912.

Stage 5 (*Fig. 5*).—Later stages show as a rule a more or less complete loss of the elongated condition. The figure is a good example of a somewhat more advanced condition, the diameter of such follicles averaging about .22 mm.

Stage 6 (*Fig. 6*).—Here we have about the maximum development of the solid follicle, before a disintegration of follicular cells begins to give rise to a lumen. The compound epithelium is from five to seven layers thick and the capsule of stroma cells is more sharply defined than ever. Such follicles have an average diameter of about .3 mm.

Stage 7 (*Fig. 7*).—At this time through the cytolysis of some of the components of the epithelium various fluid-filled cavities appear midway between the ovocyte and the periphery of the follicle. Average diameter, about .35 mm.

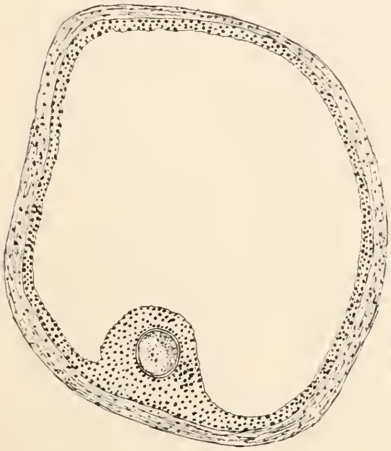
Stage 8 (*Fig. 8*).—At this stage lumen formation has made considerable progress and the follicle cells may be considered as forming two zones: a zone around the ovocyte, which is destined to form the discus proligerus and a zone occupying a peripheral position. The intervening cavity is filled with follicular fluid and cell fragments. Average diameter of such follicles, about .5 mm.

Stage 9 (*Fig. 9*).—At this time there is evinced a strong tendency for the ovocyte, with its zone of follicular cells, to occupy an excentric position due to the breaking away of the connecting strands of follicle cells on one side and the thickening by contraction of the others. This is evidently a step in the establishment of the definitive discus proligerus. Average diameter, .6 mm.

Stage 10 (*Fig. 10*).—Here we have another step in the development of the discus proligerus. Many of the largest follicles found have been in this stage of development. Average diameter, about 1 mm.

Stage 11 (*Fig. 11*).—This is what might be termed the definitive follicle. The discus proligerus is in the form of a smooth mound of follicle cells projecting into the lumen from one side of the follicular wall. The remaining part of the follicle is lined with a thin smooth sheath of follicle cells. There is a great deal of variation in the size and shape of the definitive follicle, many being flattened or otherwise distorted by the presence of various obstructions such as older follicles or dense masses of stroma cells. The average diameter of the less distorted definitive follicles is about 1 mm.

Stage 12 (*Fig. 12*).—The conditions shown in this figure are readily recognized as those typical of follicles shortly after the onset of follicular atresia. The characteristic symptoms of this process are seen in the less deeply stained follicle cells and in the tendency of the latter to wander into the lumen. Such follicles frequently show a slight or marked diminution in size, due probably to a resorption of the follicular fluid. It is in such follicles that one finds the completion of the second maturation division, a process which does not normally take place until after ovulation.



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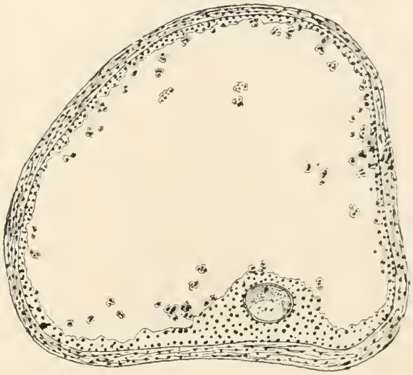

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FIG. 11. A definitive follicle with maturing ovocyte located in the mound-like discus proligerus. The lumen is filled with a clear fluid. A membranous capsule composed of stroma cells surrounds the follicular wall (×50).

FIG. 12. A follicle fully mature and entering upon process of follicular atresia. The cells of the discus proligerus and of the wall surrounding the lumen are beginning to disintegrate and to wander into the lumen (×50).

In referring stages in the development of the ovocyte or nucleus to their appropriate follicular stages it will be convenient to remember that the first twelve figures have numbers corresponding to the twelve stages of follicular development; hence it will be necessary to refer only to stage 4, 7 or 10, with the understanding that these stages are illustrated in Figs. 4, 7 and 10.

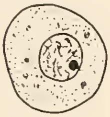
VI. Development of the Ovocyte with Especial Reference to Vitellogenesis and the Comparative Rate of Growth of Nucleus and Cytoplasm.

The ovocytes in primordial follicles (stage 1) are comparatively small cells with homogeneous cytoplasm and large nuclei, the average diameter of ten typical cells being .033 mm. and that of their nuclei .015 mm. At the beginning of the growth period then the cell diameter is only about twice that of the nucleus.

Soon after the formation of the primordial follicle, before any marked growth of the cell has occurred, distinct spherules of fatty material are distinctly visible in preparations fixed in 10 per cent. formalin and stained in Sudan III. Evidently yolk metabolism has begun at this stage. These spherules are so brightly stained with the Sudan that no other interpretation of their character is admissible. They appear excentrically, being confined to one side of the nucleus, thus indicating an early cell polarity. The average diameter of such ovocytes is about .35 mm. and that of their nuclei about .17 mm. The proportionate size of cell and nucleus, therefore, has not been materially altered. Such a cell is shown in Fig. 14, which was drawn from an ovocyte somewhat below the average in size, occupying a follicle in a condition between stages 1 and 2.

The changes in the ovocyte as found during follicular stages 2, 3 and 4 culminate in a condition shown in Fig. 15, where the cell is frequently elongated, showing polarity and bilaterality. The yolk spherules are very distinct and abundant and are confined to the pole opposite to that occupied by the nucleus. The average largest diameter of such cells is about .08 mm. and that of their nuclei about .025 mm. It will be noted that the cytoplasmic mass has increased relatively much more rapidly than has that of the nucleus, although the latter has doubled its diameter and increased its mass several times. The zona pelucida is present as a comparatively thin but dense layer, which shows evidences of having been laid down as a mesh-work of fibrous material secreted by the basal portions of the follicle cells.

During follicular stages 5, 6 and 7 a curious change occurs in connection with the process of vitellogenesis. There is a gradual disappearance of the yolk spherules (which earlier constituted such a marked feature of the cytoplasm) culminating in the condition shown in Fig. 16, in which the protoplasm of the ovocyte has acquired a secondary homogeneous structure, with a coarsely alveolar appearance. There are present scarcely any discrete fatty particles, but the whole cytoplasmic mass assumes a pinkish tint when subjected to Sudan III, a circumstance that would seem to indicate the presence of fatty materials in solution. Such a stage is evidently equivalent to that seen in *Dasyurus* and designated by Hill as the "pseudo-alveolar" stage (compare Hill, '10, Fig. 4). It will be noted that the nucleus is drawing closer to the periphery and has reached its maximum size, with a largest diameter of about .025 mm. and always somewhat flattened in form. The diameter of ovocytes of this type averages about .1 mm. The zona has attained its definitive thickness of .003 mm. and is a dense membrane showing no radiations like those which have given to the homologue of this structure in



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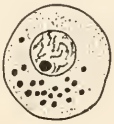

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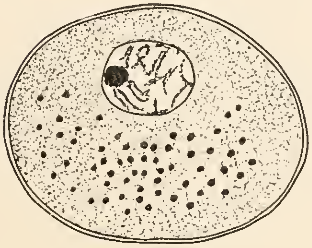

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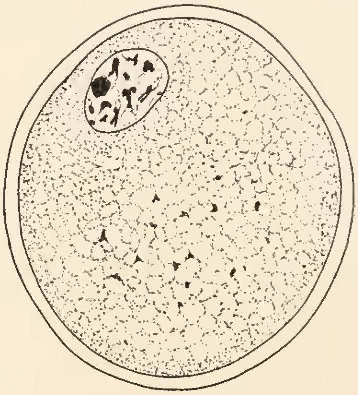

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FIG. 13. A primordial ovocyte (×410).

FIG. 14. An ovocyte at the time when an epithelial follicle has just been established. Note the presence of yolk granules (×410).

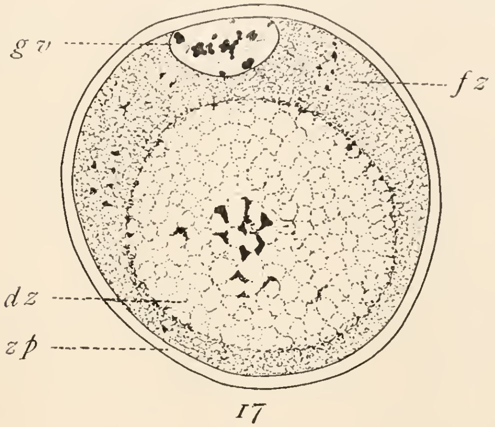
FIG. 15. A half-grown ovocyte, showing a characteristic elongated shape and the presence of numerous yolk granules (×410).

FIG. 16. An ovocyte practically full grown, in the so-called "pseudoalveolar" stage. Note that the yolk granules have almost entirely disappeared (×410).

other mammals the name "zona radiata." The cell is now over four times as great in diameter as the nucleus and there is little further alteration in their relative masses until after the rupture of the membrane of the germinal vesicle when the first maturation spindle is established.

During the stages of follicular development numbered 8, 9 and 10 there occurs a gradual reorganization of the cytoplasmic materials of the ovocyte. A zone of denser homogeneous protoplasm comes to occupy a peripheral position, forming an increasingly more and more sharply defined cortex, somewhat thickened at the point where the nucleus is flattened against the cell membrane. In the center of the ovocyte the protoplasm has assumed the character of a very coarse alveolar mass in the meshes of which are rather large irregular solid bodies which stain with Sudan III. The alveolar structure, which is seen in sections of material fixed in neutral formalin, assumes in paraffin sections the appearance of a fluid core in which are suspended scattered strands of deeply staining fibrous material and a central irregular mass of large solid pieces of irregular size and form. Such a condition finds its exact counterpart in *Dasyurus* (see Hill, '10, Fig. 1), and is characteristic of the same stages of follicular development. The condition at the time when the clearest definition is established between the two cytoplasmic zones is shown in Fig. 17. Here the "formative zone" (*f.z.*) is shown as a somewhat thicker cortical layer than is usually found, and the deutoplasmic mass (*d.z.*) is represented as coarsely alveolar, a structure which it appears to have when seen in formalin preparations. It will be noted that the nucleus occupies a position in the middle of the thickest part of the formative cortex, a position which probably represents the animal pole of the ovocyte. The diameter of the cell at this stage is on the average about .12 mm., while that of the nucleus has not changed since the stage represented in Fig. 15. The ovocyte is now full grown and ready for the changes incidental to the maturation processes.

During maturation a very radical change in the cytoplasmic structure of the ovocyte takes place. The comparatively homogeneous formative zone of the full-grown ovocyte has moved to one pole and forms a cap of considerable density, thick in the center and thinned out at the periphery. The deutoplasmic mass (*d.z.*) occupies the opposite pole and is practically in contact peripherally with the ovocytic membrane. The structure of this deutoplasmic material is represented in



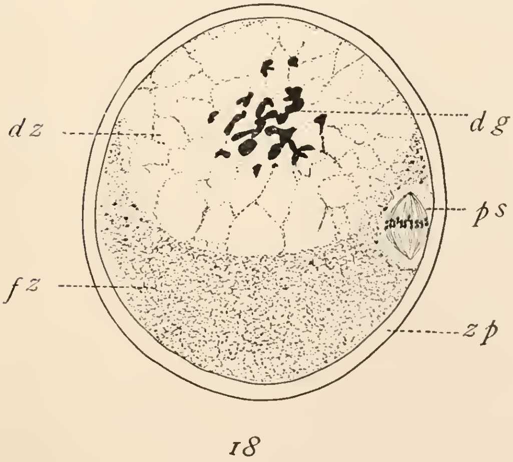


FIG. 17. A full-grown ovocyte, showing cytoplasmic organization, etc. Deutoplasmic zone (*dz*), formative zone (*fz*), germinal vesicle (*gv*), zona pelucida (*zp*) (×410).

FIG. 18. A maturing ovocyte, showing the new reversed polarity. The ovocyte is placed with the animal pole upwards. The deutoplasmic zone (*dz*) occupies the animal pole, the formative zone (*fz*) occupies the vegetative pole. The polar spindle (*ps*) lies in a tangential position at the equator of the ovocyte. Deutoplasmic granules (*dz*) lie in the center of the deutoplasmic mass. The zona pelucida *zp*) is a dense envelope, without radiations.

Fig. 18 as it appears in paraffin sections. The two zones are very sharply defined. The first maturation spindle, which one would expect to find in the thick middle part of the formative zone, occupies a position near the equator of the cell in the thinned-out peripheral portion of the formative zone that overlaps and partly surrounds the deutoplasmic mass. What is the significance of this peculiar position of the polar spindle? Exactly the same conditions are met with in the egg of *Dasyurus* and in that animal are interpreted by Hill as indications that the ovocyte has undergone a complete reversal of polarity. According to him the formative protoplasm occupies now the vegetative pole, while the deutoplasmic mass lies at the animal pole. This interpretation is borne out by the peculiar position of the spindle which occupies a position as near the animal pole as is possible without leaving the formative protoplasm or the peripheral position necessary for the extrusion of the polar body.

In view of these conditions we may well hesitate to apply any sort of phylogenetic interpretation to the "telolecithal" character of this ovum. Doubtless we have here a polarity of the germ cell which is merely incidental to changes connected with the extrusion of the deutoplasm, which I believe occurs in the armadillo in much the same fashion as that described by Hill for *Dasyurus*. The evidence for this conclusion forms the material for a subsequent paper. The armadillo ovum is to be considered as primitive not because it shows a "telolecithal" organization but because it is so nearly identical in many details with that of a number of marsupials.

With the rupture of the germinal vesicle and the establishment of the first cleavage spindle a very marked diminution in the size of the nuclear material is manifest. This is due to the loss of much fluid and perhaps some chromatin to the cytoplasm, and also to a marked condensation of the remainder, as will be shown more in detail when the nuclear phenomena come up for discussion.

VII. Nuclear Changes During the Growth Period of the Ovocytes.

The nuclear changes from the time when the ovocyte is in the primordial follicle stage till the establishment of the first cleavage spindle constitute the prophases of maturation. The earlier as well as the later changes should receive attention. Students of mammalian ovogenesis, however, have as a rule restricted their observations to the changes immediately connected with maturation divisions, ignoring the long period of nuclear and cell differentiation that leads up to and doubtless conditions these divisions. The following account makes no claim of comprehensiveness but will serve to suggest some of the more significant phases of nuclear behavior that are characteristic of this period.

The nucleus of the ovocyte in the primordial follicle (Fig. 19) shows the chromatin in the form of long, more or less coiled threads, which are sometimes so tangled as to form a pseudoreticulum. In neutral stains such as iron hæmatoxylin and copper chrome hæmatoxylin the chromatin threads and the plasmosome take the same stain, but when double staining methods are employed the difference between the two materials is clearly brought out. When neutral safranin and acid violet are used the chromatin takes the violet color from the acid reagent and the plasmosome takes only the neutral color, appearing bright red. With toluidin blue and acid fuchsin the chromatin takes a red color from the acid dye and the plasmosome is stained bluish with the basic stain. With thionin and erythrosin the chromatin takes a red color from the acid erythrosin and the plasmosome is stained purple from the thyonin. Evidently then at this period the chromatin is basic and the plasmosome acid in character. The plasmosome is also shown to be a vesicular structure containing some vacuoles or granules. It is practically certain also that the chromatin threads, each of which must be identified as an elongated chromosome, are diploid in character, since synapsis has occurred during the organogenesis of the ovary. It is only in later stages that the diploid composition of these bodies manifests itself.

In the early simple epithelial stage of the follicle no marked changes in the character of the nuclear elements has occurred (Fig. 20), except that the chromosome threads are a little shorter and thicker.

As the follicle develops through stages 2, 3, and 4 we find nuclear changes corresponding to those shown in Figs. 21, 22, and 23, which are evidently to be interpreted as stages in the separation of the closely fused diplotene threads into their component halves. Occasionally the whole complex, as in Fig. 21, is seen to be composed of elements distinctly double in character. More frequently, however, the condition is less obvious, as in Figs. 21 and 22, where the double elements have opened up into V's and rings, or occasionally have become precociously condensed into chromsomes of tetrad-like structure. There is no change as yet in the staining reactions of the nuclear components.

In connection with follicular stages 5 and 6 we customarily find in the nucleus of the ovocyte very marked shortening of the chromosomes accompanied by an increasing vagueness and irregularity of outline (Fig. 24). The diploid character of the elements is pronounced. The chromatin is undergoing a chemical change from a basic to an acid character, while the plasmosome still retains its acid affinities. This change is shown clearly when the neutral safranin and acid violet combination is used, for the chromatin no longer stains violet but assumes a faint pinkish hue from the safranin. Likewise, when toluidin blue and acid fuchsin are employed the chromatin stains bluish instead of bright red as formerly, indicating a change from basic to acid character. Slender threads of linin connect the various chromosome bodies. The chemical character of the plasmosome is not altered.

In follicular stages 7 and 8 the process of chromosome condensation has made considerable progress as is shown in Fig. 25. There are very great individual differences in the degree of condensation seen in the various units of the complex. Some of them have attained a form practically like that seen just before spindle formation, while others are still elongated and irregular. Linin threads, usually double in character, still form numerous connections between the chromatin elements. Both chromosomes and plasmosome at this stage take basic stains with about equal avidity, the latter being easily recognizable from the former by its greater size and vesicular character.

In follicular stages 8 and 9 the nucleus assumes the character shown in Figs. 26*a* and *b*, two sections through the same nucleus. Here the entire chromatin complex has undergone further condensation until each element is in the form of a double chromosome more or less clearly defined. There is a strong tendency, often much more clearly manifest than the illustration would indicate, for the larger chromatin elements to aggregate into a dense central mass and for the smaller elements, which are frequently single spherical bodies, to lie in contact with the nuclear membrane. A few of these small peripheral elements are noted to be connected by linin threads with the large chromosomes forming the central group. It is not possible to enumerate the chromosomes at this time, but a number of fairly accurate approximations have been made which would indicate that there are about sixteen large chromosomes besides a varying number of small peripheral elements. These bodies may be supernumerary chromosomes which subsequently either attach themselves to the large elements or are thrown out into the cytoplasm on the rupture or dissolution of the nuclear membrane. There are seldom any evidences of small chromosomes in the spindles during the actual maturation process, but partially formed spindles, as that shown in Fig. 29, show certain small elements attached to spindle fibers which may be homologized with the small peripheral chromatin elements of the stage of nuclear development under consideration. The plasmosome is no longer to be identified with certainty. In Fig. 26*b* is seen a large body of diploid form which stains less deeply than do the other chromosomes. This may be a stage in the transformation of the plasmosome into a chromosome, but of this I cannot be certain. In none of the nuclei examined have I seen indications of the dissolution of the plasmosome and am inclined to the view that it is the equivalent of a heterochromosome. Another point which is obvious in the figures is the increasing irregularity of the nuclear membrane. It is evidently becoming very thin and is losing its turgor, as the wrinkles in its surface indicate. This could hardly be due to shrinkage in fixation, for the nuclei of earlier stages retain their spherical contours. Moreover the wrinkles occur chiefly on the side of the nucleus toward the cell membrane to which is in close proximity at this time.

In follicular stage 10 the nuclei of ovocytes are in a condition represented by Figs. 27*a* and *b*. These two drawings indicate the entire chromatin content of a single nucleus. It will be noted that the chromosomes are clearly defined but still massed in groups. There is no distinguishable plasmosome. All of the chromatin takes with equal avidity basic dyes and must therefore be considered rich in nucleinic acid. There are only remnants of the linin network in the form of scattering slender threads running from the chromosomes to the periphery of the nucleus. A number of these threads appear to converge at one point and may indicate the first steps in spindle formation. Subsequent stages deal with the actual maturation divisions.

VIII. The First Polar Spindle.

The stages immediately preceding the establishment of the complete cleavage spindle are difficult to find in sections, owing probably to the great rapidity of the process. One of the few stages that have come to light is shown in Figs. 28*a* and *b*. In this case it is obvious that the spindle is forming, but chiefly at one end. The chromosomes are very clearly defined and have lost the tendency to be grouped into masses. There is a marked difference in the size of the individual elements, some being many times the volume of others. This is interesting in view of the fact that in the fully formed spindle there is no marked size difference among the chromosomes. The explanation of this condition probably lies in the inequality of state of condensation in the various elements. Numerous camera drawings of the chromosome complex of this period have been made, and on the basis of such drawings chromosome counts have been attempted. It is extremely difficult, however, to get even approximately correct estimates of the actual number of univalent and bivalent elements present. It seems quite obvious from examination of the figures that both single and double elements occur side by side, and it is not always possible to distinguish one type from the other. A study of Figs. 28*a* and *b* will reveal some elements very clearly tetrads and others that are clearly bivalent though not as yet tetrapartite. The small peripheral chromosomes of earlier stages are still in evidence. Some of these show no tendency to take a position in the spindle and are evidently destined as a contribution of the nucleus to the cytoplasm on the dissolution of the nuclear membrane. That this extrusion of chromatin into the cytoplasm actually occurs is evidenced by the presence in the cytoplasm of mature ova of minute nucleus-like bodies of cytoplasmic chromatin.

The spindle is evidently completely established within the nuclear membrane as one must conclude from the occasional occurrence of such appearances as that shown in Fig. 29. In this case the membrane is exceedingly delicate but still unmistakable. When the membrane finally disappears there is evidently cast out into the cytoplasm a large amount of material, largely fluid, but probably partially solid in character. With the loss of this liquid the spindle shrinks in size and the chromosomes undergo marked condensation, as must be evident from comparison of these elements in Figs. 29 and 30 which are drawn to the same scale. Still further condensation of chromatin seems to occur during the metaphase as a comparison of Fig. 34 will show. The late prophases show the chromosomes as bivalent elements, occasionally having the appearance of typical tetrads (Figs. 31 and 32). The spindle is apparently a naked central spindle without mantle fibers or asters and is evidently a self-contained system insulated from the cytoplasm by a sheath of inert material, coarsely vacuolated, a material which may consist largely of the extruded nuclear sap which is in equilibrium with the surrounding cytoplasm, and through which no metabolic exchanges between nucleus and cytoplasm can occur.[1]

In equatorial plate views of the metaphase one can frequently count the chromosomes with every assurance of accuracy. Fig. 32 is a typical equatorial plate view of the first maturation spindle and one can judge from this example as to the feasibility of enumerating the chromosomes. By far the most frequent count obtained is that of 16 diploid elements, but some counts show as few as 14 and others as high as 19. The larger number may be, and probably is, due to the precocious separation of a few of the double elements in the early anaphase. The lower count may be due to some elements being fused with or hidden under others. Personally I am convinced that the normal reduced number of chromosomes in the female of this species is 16, since this number has occurred as often as all others combined.

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[1] These conditions accord with those described by F. R. Lillie for certain phases of maturation in the egg of Nereis.

Fig. 34 shows a rather unusual spindle in which all of the chromosomes are in the form of well-defined tetrads, in some cases just separated into diads. A number of other spindles have been found where some of the elements were typical tetrads of this sort, but others were merely double in appearance. In the anaphases, a good view of which is given in Fig. 35, the chromosomes are clearly diads of dumbell shape. In late anaphases, as in Fig. 36, these diads assume the flattened shape characteristic of the tetrads of the prophase and form ring- or disc-shaped masses at either end of the barrel-shaped spindle. The band-shaped *Zwischenkörper* forms a characteristic feature of this phase of the division.

During the prophases and anaphases of polar body formation the spindle lies in a parallel or tangential position with reference to the cell membrane and it is only in the last steps of the anaphase that any sign of polar extrusion appears. The polar body begins to form after the manner shown in Fig. 36, by the appearance of a slight furrow, like the beginning of a cleavage furrow. As this furrow deepens the spindle assumes a position more nearly perpendicular to the surface of the ovocyte and the polar body is constricted off as in Fig. 37.

IX. THE FIRST POLAR BODY AND THE SECOND POLAR SPINDLE.

Only eight ovocytes with one polar body have been found in the present study as compared with literally hundreds with first polar spindles. This fact would seem to suggest that the majority of the ovocytes come to an equilibrium in the metaphase of the first maturation division, and require some special stimulus to cause them to complete this division. It is very probable that the stimulus needed is that brought about by mating. If we may infer from analogy with other mammalian studies of ovulation it is likely that follicular rupture takes place immediately after the ovocyte has extruded one polar body; hence all of the cases observed in which one polar body has been formed must be considered as ovocytes ready for ovulation. As a rule the first polar body as it appears in ovarian ovocytes is much compressed between the zona and the cell membrane, as in Figs. 38 and 41, and is some distance from the second maturation spindle. This position is, I believe, not to be interpreted as due to a migration of the ovocytic nucleus in the interim between the two maturation divisions, but rather as a shifting of the freed polar body. Observers of living mammalian ovocytes, notably Long and Mark ('11), indicate that there is a considerable space between the ovocyte membrane and the zona after the first polar body is extruded. This space would offer the necessary conditions for any shifting in the position of the polar body with reference to the site of the maturation spindle. In Fig. 40 is shown a case where the second polar spindle has established itself in close proximity to the first polar body, in which the nucleus has remained in a comparatively solid state. This is not a usual condition, however, for in practically all other cases the first polar body shows signs of a tendency to undergo mitotic division. I have never observed a well-defined spindle in a polar body, but radiating fibers are always present and the chromosomes to some extent divide and pass to two poles of the cell, as in Fig. 38. In only one case have I observed a complete division of the first polar body and that is in a decidedly atypical case shown in Fig. 42 and which is discussed in a subsequent connection. The division of the first polar body must then be considered as merely an abortive attempt at a division equivalent to the second maturation division. It seems likely that the chromatin subsequently goes back into a vesicular nucleus like that shown in Fig. 40, or like that in the fertilized egg shown in Fig. 44.

The second polar spindle can be identified with assurance only in ovocytes in which the first polar body can be observed, and as was indicated, these conditions are comparatively rare. Yet we have several exceptionally good examples of such spindles, notably those shown in Figs. 38, 39, 40 and 41. As a rule the second spindle is noticeably smaller than the first, but the difference is not nearly so obvious as is the case in the mouse egg. Apart from the lack of typical tetrads in the second spindle the chromosomes have the same general appearance as those of the first. As an illustration of this similarity of chromosomes compare Figs. 32 and 39, which are equatorial plate views of the chromosomes of first and second polar spindles respectively. The spindle shown in Fig. 41 differs from any other, either first or second, that has come under my observation in that it appears to have a set of mantle fibers in addition to the central spindle. This, however, is not to be considered typical for second maturation spindles in this species.

All of the conditions thus far described have been found in follicles of normal structure, in which there are no evidences of follicular atresia, and are, therefore, to be considered as strictly normal.

X. The Second Polar Body and the Female Pronucleus.

My observations of the second polar body are limited to three cases, the anomalous case described on the following page and illustrated in Fig. 42; the normal case shown in Fig. 43; and the fertilized egg, Fig. 44. The normal case from which the drawing (Fig. 43) was made occurred in a follicle like that shown in follicular stage 11. The section cuts tangentially across one part of the formative zone and just shaves a thin slice from the deutoplasmic mass, shown in the form of coarse vacuoles. The second polar body appears to be somewhat larger than the first, in this respect resembling the egg shown in Fig. 42. The chromosomes of both polar bodies are well scattered and there are evidences of an attempt at mitotic division. The female pronucleus is in a condensed condition, but the individual chromosomes are distinguishable. Surrounding the mass of chromatin is a capsule of homogeneous protoplasm, which probably insulates the nucleus from its own cytoplasm.

XI. An Anomalous Case of a Third Polar Spindle.

This case came to my attention very early in the investigation and was at first interpreted as a practically certain case of a parthenogenetic first cleavage. In view of recent discoveries of many examples of parthenogenetic cleavage it now appears that this spindle in no way resembles a cleavage spindle, for the egg is not in a condition for cleavage, in that the deutoplasmic mass is still an integral part of it. The only alternative explanation that occurs to me is that we have here a rare case of a continuation one step further than is normal of the processes involved in maturation. The spindle in this egg is as perfect as any polar spindle observed in my material and contains without question 16 chromosomes. It is a naked central spindle without any traces of the aster radiations characteristic of true cleavage spindles. As though in physiological sympathy with the egg cell both polar bodies are seen to have proceeded somewhat further in their development than those in any other egg observed. The first polar body has completely divided into two ootids, and the second polar body, which is very large and well-formed, shows a polar view of a mitotic spindle, homologous with that seen in the egg itself, and has likewise 16 chromosomes. This curious egg was found in a follicle like that shown in Fig. 12, in which the process of atresia had made noticeable progress. The case is unique in the annals of biology and may possibly be explicable on some other basis than that which I have suggested. Personally I see no alternative explanation.

XII. Fertilization.

Although diligent search has been made through large numbers of ovaries with fallopian tubes attached, only one tube egg has been found. This one egg, however, is so evidently a normal example of the conditions typical for the species that it warrants a detailed description. The egg is found in a part of the fallopian tube just where it straightens out in its course toward the uterus. It lies free in the tube surrounded by a coagulum of material evidently composed of the disintegrated fragments of granulosa cells. It runs through twelve serial sections of 10 microns thickness and is therefore about .12 mm. in diameter, or a little smaller than the average full grown ovarian ovocyte. The zona is well defined and unbroken. The two polar bodies, each with its nucleus in a resting phase, are situated in contact with the formative protoplasm at some distance from the pronuclei.

The male and female pronuclei are in contact and are contained within the main body of the formative protoplasm and are closely similar in size and in the condition of their chromatin, which is peculiar in that each chromosome appears to be a small vesicle, connected with others by means of linin fibers. A large plasmosome is present in each pronucleus. The cytoplasm does not show so clear a demarkation between formative and deutoplasmic zones as is usually seen in the maturation stages, but this is probably due to the fact that the plane of section is equatorial and therefore unfavorable for showing polar differentiation of any sort.

This account of a single case of fertilization might be considered a somewhat meager basis for an account of so important a process, but it is the best that can be offered at present. It is my conviction that the discovery of even one such stage is a fortunate circumstance, in view of the fact that we are dealing with wild animals captured at night and not operated on until the following morning at the earliest. Only rarely is it possible to obtain the females so soon after capture as this. The uterus associated with the ovary in which this fertilized egg was found was somewhat swollen and congested and was supposed to contain an early blastodermic vesicle, but on examination was found to be non-pregnant. No other ova were found in the fallopian tube, either proximal or distal to the cite of the one under discussion. There is a single medium-sized corpus luteum. These facts demonstrate that only one ovum is given off and fertilized at one time, and add confirmation to the contention that during the early stages of embryonic development in the armadillo the conditions are those of a single fertilized egg and a single blastodermic vesicle and that the separation into four embryonic rudiments is a process of asexual multiplication, whose *visible* manifestation comes comparatively late in the development of the blastodermic vesicle.

Summary and Conclusions.

1. A study of the ovogenesis of the armadillo reveals nothing unique except that the cytoplasmic polarity of the mature ovocyte and its genesis is practically identical with that of the

marsupial *Dasyurus*. In the sense that the ovum is like that of a member of a lower sub-class of mammals it may be considered as probably the most primitive Eutherian ovum on record. The deutoplasmic material is at first centrally situated within a capsule of formative protoplasm, but, coincident with the onset of maturation, a shifting of materials takes place, so that the deutoplasmic material is aggregated at the animal pole of the ovocyte while the formative protoplasm forms a cap-shaped mass at the vegetative pole. The first polar spindle occupies a position as near the animal pole as it can without leaving the surface of the cell or the formative material. The earlier condition may be called "centrolecithal" and the later, "telolecithal," though these terms probably imply homologies that do not exist.

2. Previous studies of mammalian ovogenesis are confined to three orders of mammals, Rodentia, Cheiroptera and Carnivora, all of which are rather highly specialized orders, according to modern systems of classification. The present study of conditions in the armadillo is the first contribution to our knowledge of the germ cells of the Edentata, and thus we may add not only a new order to the short list of those studied, but probably the order showing the most primitive conditions.

3. Since it had not been found feasible to breed the armadillos in captivity our knowledge of the maturation processes depends entirely upon studies of the ovarian ova, normal and atretic. Nine tenths of the process as here described takes place in normal follicles and hence must be considered as strictly normal. A large number of ovaries have been sectioned in order to secure the stages described in the present history. Abundance of material is necessary because some of the stages are of exceedingly rare occurrence.

4. A study of the developmental history of the follicle shows that there is no basis for the idea expressed by Rosner that the four embryos are derived from the fusion of four adjacent follicles and the coöperation of their four ova to form a compound blastodermic vesicle.

5. The full grown ovum is about .12 mm. in diameter. It is smaller than that of the cat and larger than that of man.

6. The first polar spindle and first polar body are in no way radically different from those described for other mammals. The

chromosomes appear to afford exceptional opportunities for enumeration. The haploid number appears to be 16 and the diploid, 32. There are numerous apparent exceptions, but these are the most commonly appearing numbers.

7. The second polar spindle and second polar bodies are of rare occurrence, but those studied are in no way different from those of other mammals.

8. An exceptional case of what appears to be a third maturation division is figured and discussed.

9. A single tube egg in a good state of preservation shows a late stage in the fertilization process. Both polar bodies are present, and the male and female pronuclei are large vesicles practically ready for fusion. This one case is adjudged to be typical and, on the basis of its discovery, we are in a position to add another item to the evidences already published that the quadruplets of the nine-banded armadillo are derived from a single fertilized egg. The case also serves to eliminate from further consideration all suggested explanations of the underlying basis of polyembryony that involve the idea of polyspermy.

Hull Zoölogical Laboratory,
University of Chicago,
May 1, 1912.

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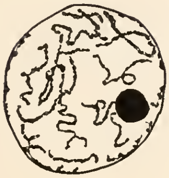
EXPLANATION OF PLATES.

PLATE I.

FIGS. 19 to 25 (inclusive) show seven successive stages in the development of the nucleus of ovocytes during the growth period (× 1,600).



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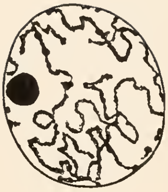

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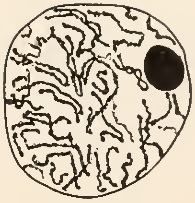

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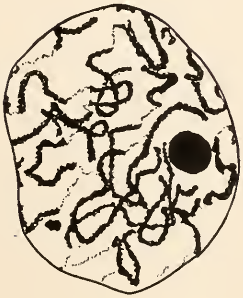

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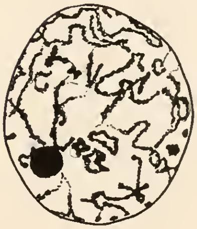

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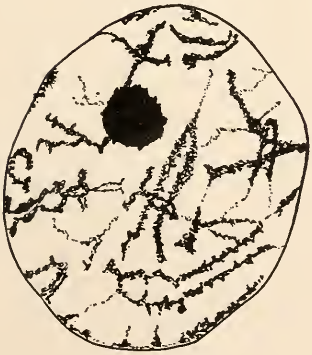

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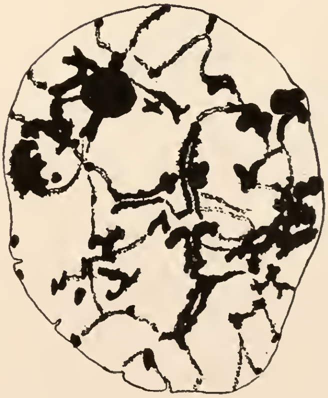

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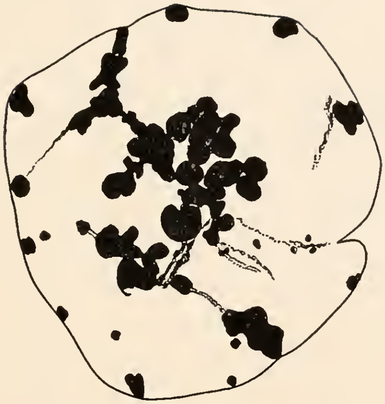
PLATE II.

FIGS. 26 (*a* and *b*) represent two sections through the germinal vesicle of an ovocyte in a follicle like Fig. 9. (×1,600).

FIGS. 27 (*a* and *b*) show two sections through the germinal vesicle of an ovocyte in a follicle like Fig. 9 (×1,600). Note the first indications of spindle fibers.

FIGS. 28 (*a* and *b*) show two sections through a germinal vesicle of an ovocyte like that represented in Fig. 11. Note that considerable progress has been made in the establishment of the first polar spindle and that the bivalent chromosomes are of very unequal size and form, some having a distinct tetrad form (×1,600).



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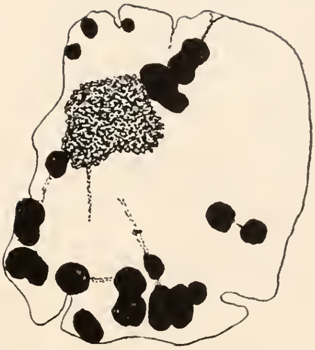

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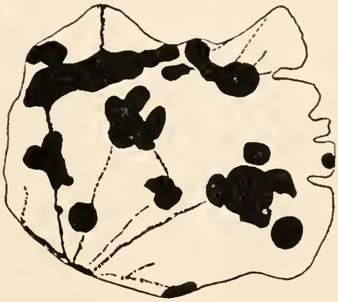

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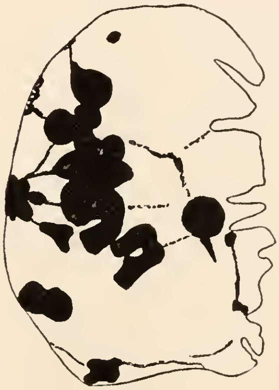

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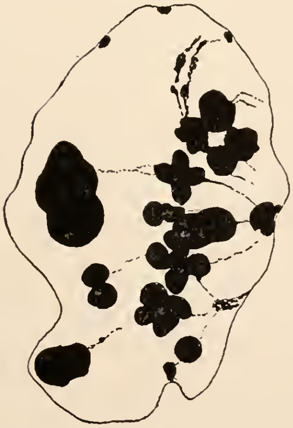

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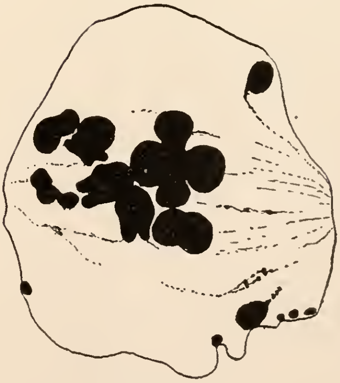

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PLATE III.

FIG. 29. A newly formed first polar spindle still within the membrane of the germinal vesicle (×1,600).

FIGS. 30 and 31. Two first polar spindles in late prophases (×1,600).

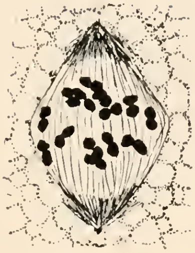
FIG. 32. An equatorial plate view of the chromatin complex of a metaphase of the first polar spindle (×1,600).

FIG. 33. A portion of an ovocyte showing a diagonal section through the equatorial plate of a metaphase stage of a first maturation spindle. Note the capsule of hyaline protoplasm surrounding the spindle, insulating it from the cytoplasm (×800).

FIG. 34. An unusually fine first polar spindle in the metaphase or very early anaphase. The chromosomes are all typical tetrads (×1,600).

FIG. 35. A middle anaphase of a first polar spindle, showing the diads as dumbbell-shaped bodies. Faint indications of centrosomes are visible. Compare the shape of the diads with that of those in the next figure (×1,600).

FIG. 36. A late anaphase of a first polar spindle. Note the changed shape of the diads, the bands-like *Zwischenkörper*, the insulating protoplasmic capsule divided into two, and the cleavage furrow destined to cut off the polar body. The position of the spindle with reference to the periphery of the ovocyte is typical (×1,600).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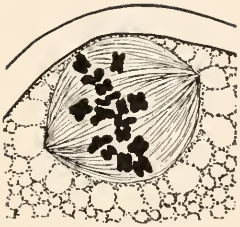
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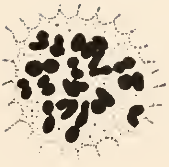
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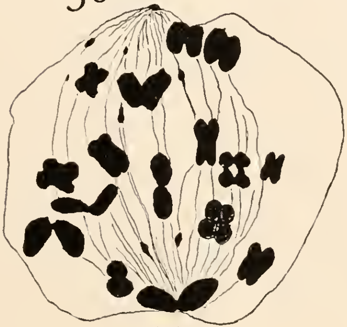
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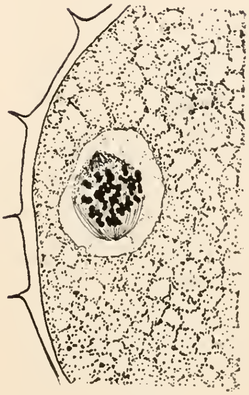
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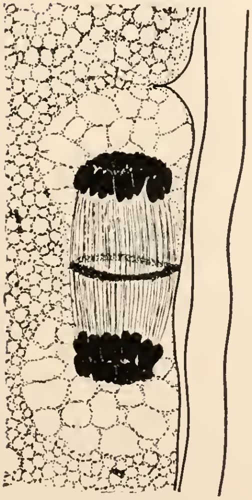
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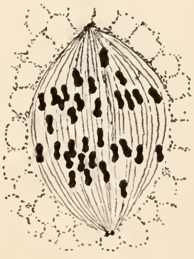
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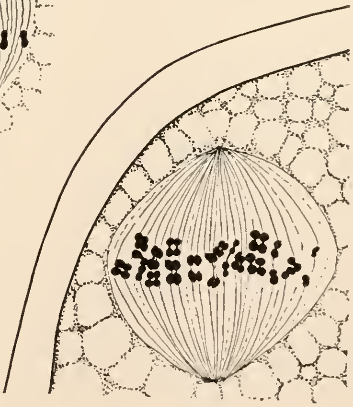
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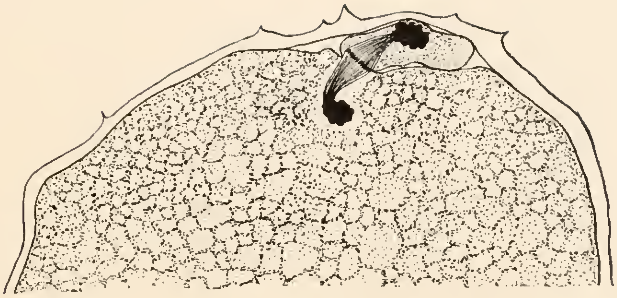
PLATE IV.

FIG. 37. Portion of an ovocyte showing the formation of the first polar body (×800).

FIG. 38. Portion of an ovocyte showing the first polar body undergoing an abortive attempt at division, and an equatorial plate view of the second polar spindle (×800).

FIG. 39. A high power drawing of the chromosome complex of the second maturation spindle shown in Fig. 38 (×1,600).

FIG. 40. Portion of an ovocyte with first polar body in a resting state and a good side view of the second polar spindle (×800).



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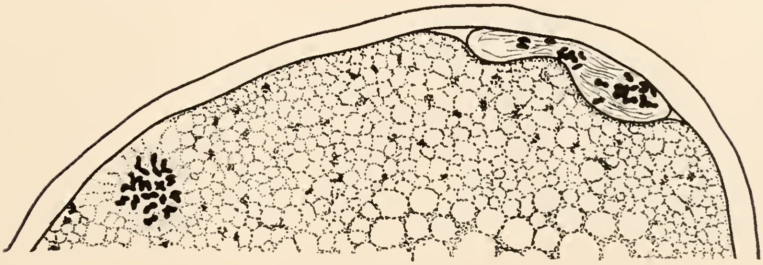

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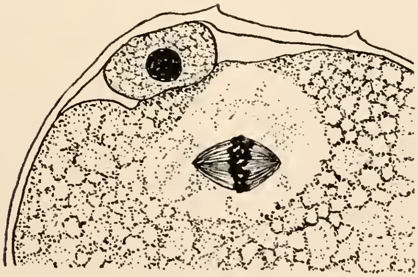

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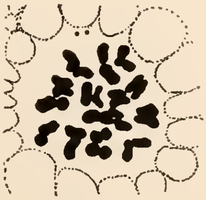

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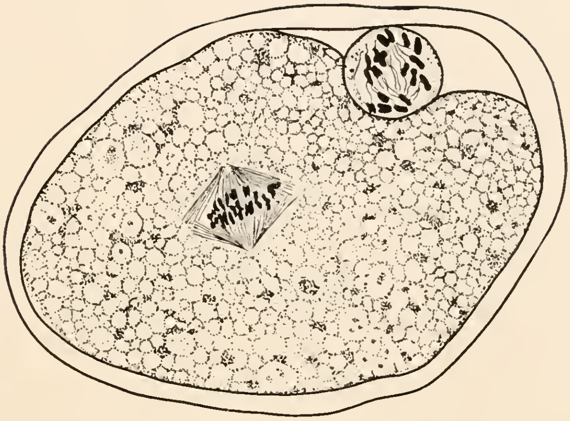
Plate V.

Fig. 41. Section through an ovocyte with a dividing first polar body and a second polar spindle of unusual character, with mantle fibers. The spindle is in an early anaphase (×800).

Fig. 42. A section through one end of an ovocyte showing three polar bodies and a third maturation spindle. The first polar body has divided, the second polar body is dividing. The egg is somewhat flattened by shrinkage but the zona pelucida is intact (×800).



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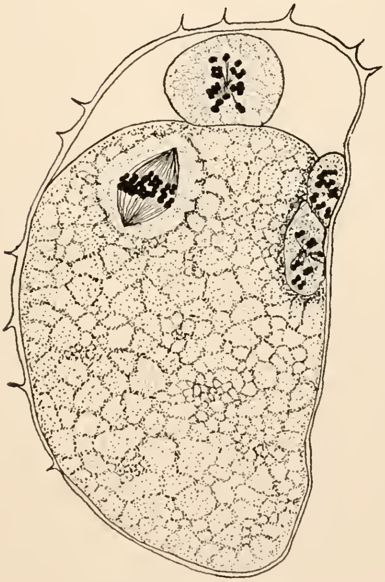

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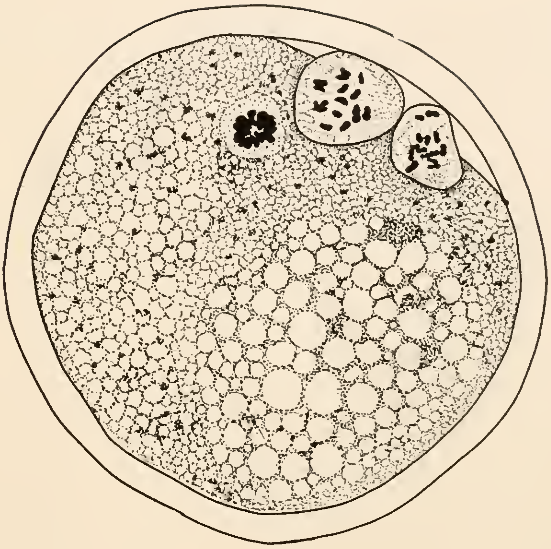
PLATE VI.

FIG. 43. A section through a mature egg with two polar bodies and a resting female pronucleus (×800).

FIG. 44. View of a fertilized egg found in the fallopian tube. Note the two polar bodies with their resting nuclei and the male and female pronuclei in contact, with their chromosomes in a vesicular or reticular condition. The drawing is a reconstruction of three sections through the formative protoplasmic cap (× 800).



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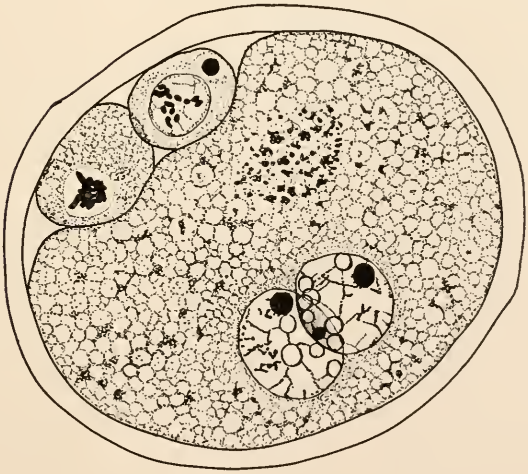

44

H. H. NEWMAN.

Vol. XXIII. *August, 1912.* *No. 3*

BIOLOGICAL BULLETIN

THE INTERSTITIAL CELLS AND THE SUPPOSED INTERNAL SECRETION OF THE CHICKEN TESTIS.[1]

ALICE M. BORING.

The problem presented in this paper was suggested to me by Dr. Pearl in connection with some work of his on the time when the secondary sexual characters first appear in the domestic chicken. The domestic chicken has a long list of secondary sexual characters. Will the cytology of the reproductive organs throw any light on the cause of the development of these secondary characters? The observations described in this paper deal with the cytology of the testis, with especial reference to the problem of secondary sexual characters.

The problem of the cause of the secondary sexual characters has been approached in many ways, but the favorite one is the internal secretion theory, that is, that the secondary sexual characters are dependent on a secretion formed by the cells of the primary reproductive organs. Some phases of this theory consider the germ cells themselves as the source of this secretion, but oftener the so-called interstitial cells are regarded as the secreting agents. We find frequent references to these interstitial cells in the literature, but there has been great confusion between the terms "interstitial tissue" which might be any connective tissue lying between the seminal tubules, and the term "interstitial cells," which by some workers is used for gland cells among the connective tissue between the tubules. Workers disagree as to whether these gland cells are epithelial or mesenchymous in origin. Another term frequently used is "interstitial gland" which seems to be used for any large accumulation of the gland cells in the interstitial tissue. Poll, in his studies of hybrid birds, speaks of the increased development of the interstitial gland composed of cells of epithelial nature in contradistinction to the connective tissue. Ceni, in his account of experiments with feeding ducks on caffeine and removing the cerebrum, speaks of an increase of the amount of interstitial tissue at the expense of the cells of the seminal tubules and mentions groups of epithelial cells called interstitial glands. On the other hand, Loisel distinctly states that he finds no evidence of activity in the cells of the interstitial tissue of birds at any time of year, but finds a fatty secretion formed by the Sertoli cells. Mazzetti finds no more evidence of secretory granules in the cells of the interstitial tissue than in the cells of the seminal tubules. He claims that interstitial cells are derived from connective tissue cells and gives an interesting list of variations in the number of interstitial cells in different animals and in the same animal at different times of year. Kirkbride states that the number of interstitial cells in the testes of newborn infants is very variable. Gudernatsch describes a case of hermaphroditism in man where the interstitial tissue of the testicular portion of the ovotestis was enormously developed, and yet the secondary sexual characters were such that the individual was usually regarded as a female.

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[1] Papers from the Biological Laboratory of the Maine Experiment Station, No. 38.

So the question of the origin, nature and function of the interstitial cells has been by no means settled. Now if it is a general law that the interstitial cells and secondary sexual characters are causally interrelated, one would certainly expect to find abundant interstitial cells in a bird with such marked secondary sexual characters as the domestic chicken.

METHODS.

Just-hatched chicks were used first, as there are some indications that there may be a differentiation in respect to certain secondary sexual characters at that early age. The chicks were all Barred Plymouth Rocks from the Maine Experiment Station stock, not a day old. The gonads are easily cut off from the surface of the kidney with scissors. They were fixed in Gilson's, Flemming's and Hermann's solutions, these two osmic agents being used especially because the internal secretion of the testis has usually been described as appearing in the form of fat or oil drops in the cells.

For comparison with these, I have studied the testes of fourteen older birds, ranging from 5 to 12 months in age. These were all but three pure Barred Plymouth Rocks, one was a White Leghorn, and two were cross-breds. They were killed at different times of year, October, November, January, March and April. In birds over six months of age, dividing germ cells were found, all stages from spermatogonia to spermatozoa. The testes from six of these birds had been put up previously for other purposes for which the best cytological methods were not necessary, but the general observations on these accord so well with those on the very best fixed material, that I do not hesitate to state that my observations cover fourteen birds. The other eight testes were cut in small pieces and fixed in Gilson, Flemming and Hermann. With two birds, every possible precaution to secure absolutely normal fixation was taken: the testis was removed from the living bird and put directly into Flemming, heated to the temperature of the bird's body, and the testis was then cut into thin slices with a razor, as it was held in the hot Flemming.

The ordinary method of clearing in xylol for embedding and mounting was not used with all of the material, as xylol dissolves fat. From Mallory and Wright's "Pathological Technique," I found that chloroform and clove oil do not dissolve fat fixed in osmic acid, so by clearing the Flemming material in chloroform or clove oil, and mounting the sections in chloroform balsam, I got preparations showing fat.

Observations.

In sections of the just-hatched testis, one can see without great magnification that about half the substance is interstitial connective tissue (Fig. 1). The seminal tubules are small and far apart. Careful detailed study shows that the cells of the seminal tubules have comparatively large round nuclei, with a linin network and scattered chromatin granules; in fact, they are typical resting germ cells. There were no signs of any dividing cells, or of any spermatocytes or spermatozoa. These cells are evidently quiescent early spermatogonia.

The abundant interstitial substance shows no marked differentiation. It looks like embryonic connective tissue (Fig. 2). The fibers run in all directions. The nuclei are mostly round, but some of them are elliptical or irregular in shape. However, the nuclei in the connective tissue sheath of the testis are all typical elliptical connective tissue nuclei. Why this difference? One cannot help noticing that the texture of the interstitial

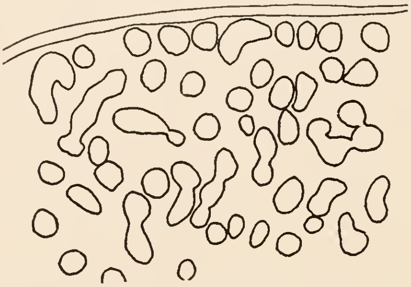

FIG. 1. Section through testis of just-hatched chick, showing seminal tubules in outline, interstitial spaces, and testis sheath. × 100.

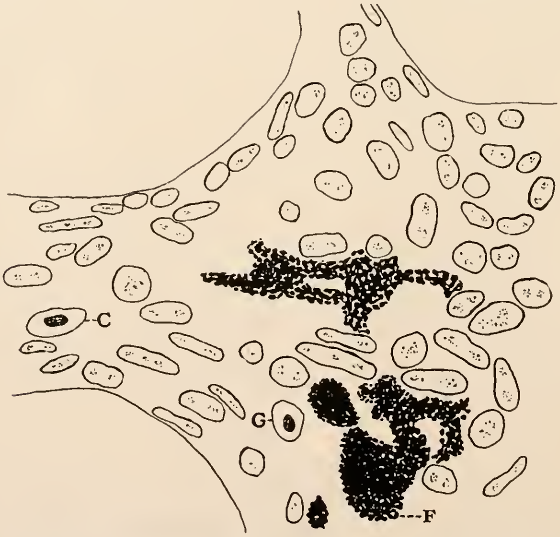

FIG. 2. Portion of Fig. 1, showing nuclei and fat (*F*) in interstitial tissue. *C* = blood corpuscles. × 1,000,

substance where the nuclei are mostly round is loose and reticular, while the fibers of the sheath lie parallel and close together, and look as though they were stretched tight around the testis. This suggests that the difference in the shape of the nuclei may be due to difference in pressure relations.

Further, none of these cells of the interstitial substance show any signs of glandular activity. The so-called "interstitial cells" have been described in a recent paper by Mazzetti as grouped in complexes in the intercanalicular spaces, as polyhedral or ovoid with abundant protoplasm, large round nucleus, an evident nucleolus, protoplasm in two zones, a clear peripheral and an intensely-coloring central, also as containing secretory granules, either crystalline or fatty in nature. There are no such cells in the interstitial substance of the testis of just-hatched chicks, and the connective tissue cells which make up this substance show no sign of glandular activity. In sections of material cleared in clove oil so as not to dissolve fat, great masses of fat stained black by osmic appear in the interstitial tissue, but they are not grouped about any especial cell, and have no appearance of being secreted by any of these cells (Fig. 2).

This being the condition in the quiescent testes of the just-hatched chick, we may next compare with it the testes of birds in which active spermatogenesis is going on.

In the older birds, there is a striking individual variation in the size of the seminal tubules. This variation has no relation to breed, age, time of year, or apparent stages of the germ cells, but occurs in birds of the same breed, and age, killed on the same day, with the germ cells in both showing all stages of spermatogenesis. However, there is one constant relation, the relation of the size of the seminal tubules to the amount of interstitial tissue. Wherever the seminal tubules are small the interstitial spaces are wide, and where the seminal tubes are large, the interstitial spaces are narrow. Figures 3 and 4 bring out this point. It is not only the width of the spaces between any two tubules that varies but also the size of the triangular spaces where any three or four tubules come into juxtaposition. This relation can be proved by accurate measurements.

For these measurements, I used slides from the testes of four Barred Plymouth Rock cockerels of about the same age, killed on the same day, all in active spermatogenesis. The measuring was done with an eyepiece micrometer. One hundred seminal tubules were measured in each of the four birds, and 100 spaces between tubules. There seems to be no accurate way to measure the triangular spaces.

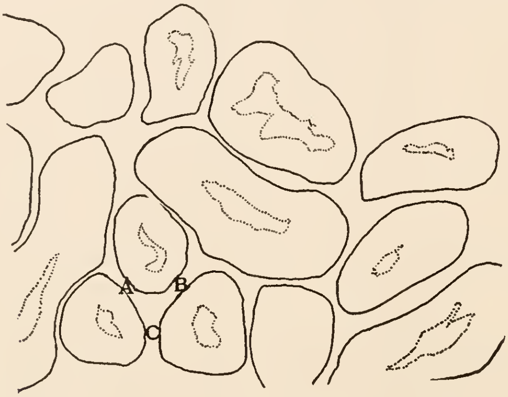

Fig. 3. Section of testis of ♂ 666, showing small tubules and wide spaces. × 100. *ABC* = one triangle in which the cells were counted, such as Fig. 5 represents.

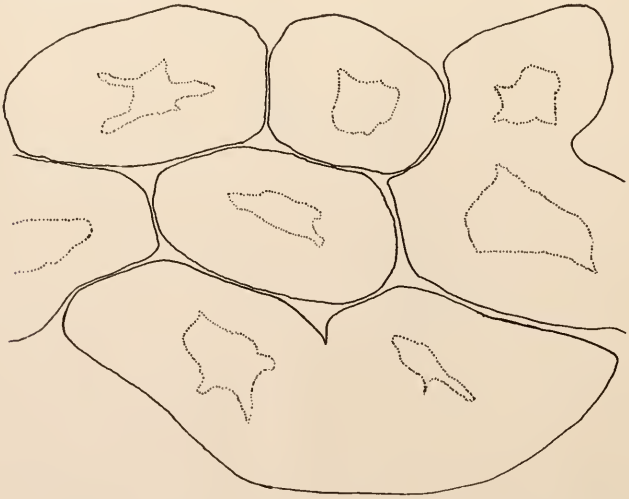

Fig. 4. Section of testis of ♂ 147, showing large tubules and narrow spaces. × 100.

A glance at Table I. will show the relation between the two sets of means: taking the birds in the order nos. 666, 1271, 2323, 147, the tube size increases, and the space width decreases.

TABLE I.

ABSOLUTE MEASUREMENTS AND CELL COUNTS OF TESTICULAR ELEMENTS. MEAN VALUES.

Bird No.	Tube Width.	Space Width.	No. Cells in Triangle.	No. of Long Cells.	No. of Round Cells.	No. of Light Cells.	No. of Dark Cells.	No. of Dark Long Cells.	No. of Dark Round Cells.
666	45.43	3.46	67.68	33.16	34.52	59.00	8.68	3.28	5.40
1271	50.82	3.015	60.76	27.32	23.44	58.92	1.84	.24	1.60
2323	82.99	1.455	23.16	14.92	8.24	20.60	2.56	.80	1.76
147	93.14	1.385	50.96	33.68	17.28	50.08	.88	.48	.40

TABLE II.

RELATIVE PROPORTIONS OF DIFFERENT CELL ELEMENTS.

Bird No.	Per Cent. Long Cells.	Per Cent. Round Cells.	Per Cent. Light Cells.	Per Cent. Dark Cells.	Per Cent. Dark Long Cells	Per Cent. Dark Round Cells.
666	48.98	51.02	87.17	12.83	37.79	62.21
1271	44.96	55.04	96.80	4.20	13.27	86.73
2323	64.42	35.58	88.94	11.06	31.25	68.75
147	66.09	33.91	98.27	1.73	54.54	45.46

On examining the interstitial spaces in these four testes with greater magnification, there appears to be what one would naturally expect, a greater number of cells in the larger triangles and wider spaces. An accurate count of the cells shows this to be the fact. The cells in twenty-five triangles of each of the same four testes were counted. In order to count over comparable areas the cells were counted from the points of the triangle along the spaces between each two tubules to about half way to the next triangle (Fig. 3, *A*, *B*, *C*). The sections in the four testes were of course cut the same thickness. A study of Table I. shows that the number of cells varies with the width of spaces and inversely as the size of the tubules, with one exception. That is, arranged according to mean number of cells in spaces, the birds stand in the order 666, 1271, 147, 2323, as contrasted with 666, 1271, 2323, 147, the order for tubule size. The exception is in the reversal of 147 and 2323. The explanation of this lies in the fact that the measurements are of the spaces between two tubules and the count of cells includes the triangular space between three tubules. There is very little difference in the space width of 147 and 2323, but the triangles of 2323 are decidedly smaller.

This difference in tubule size is so striking and seems so strange in birds so nearly alike in other ways, that I tried to find any possible other explanation than that of individual variation. It occurred to me that possibly the size of tubules might vary in different parts of the testes, and as the pieces of testes first sectioned were cut out of the organ without any reference to position the probability is that they were from different parts in the four different testes. However, in two birds killed, I carefully fixed pieces from the periphery and from the center of the organ, as these two regions would represent any difference in pressure conditions or in growth. The sections of these pieces show that the tubule size is the same throughout. Though the testis from which Fig. 3 is drawn has many fully formed spermatozoa, it is just possible that it has not reached the climax of growth, and would eventually have contained tubules as large as those in Fig. 4.

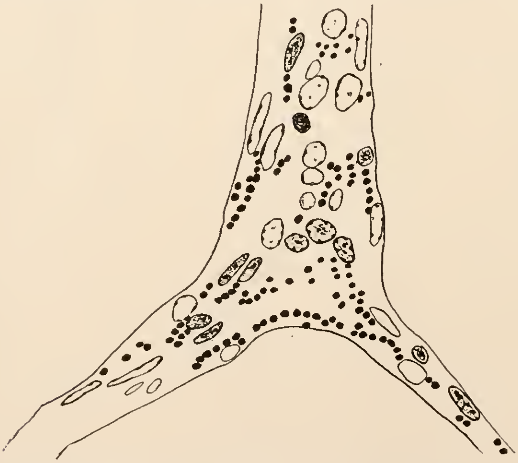

FIG. 5. Triangular space of interstitial tissue in ♂ 666, showing many nuclei of different shapes and staining capacity, also many fat globules arranged in rows. × 1,000.

In the adult testis, the nuclei vary in shape exactly as in the just-hatched chicks, and there are here also no cells differentiated from the connective tissue fibers. In the adult there are more elongated nuclei and fewer round ones. In the testis, where the tubules are large, and crowded close together, so that there is not much interstitial tissue between them, the nuclei are mostly elliptical, with some round ones in the triangle (Fig. 6). Where the tubules are small and far apart, leaving much interstitial tissue there are more round nuclei, and they lie not only in the triangles (Fig. 5). In counting the nuclei as mentioned above, I differentiated between long and round ones. Table I. shows the mean number of each kind, and Table II. gives the percentage of each shape to the total number of nuclei. In ♂s 666 and 1271, with the small tubules, 48 per cent. and 44 per cent. nuclei are long, but in ♂s 147 and 2323 where the tubules are large, 66 per cent. and 64 per cent. are long. This relation must mean a difference in mechanical conditions of pressure.

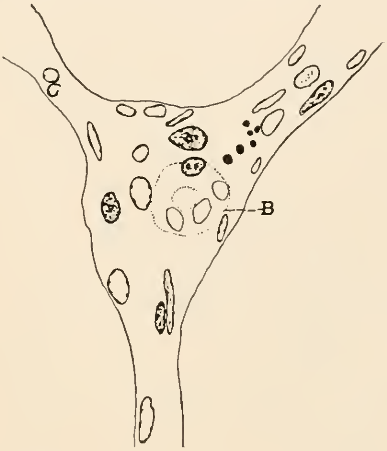

FIG. 6. Same as Fig. 5, but from ♂ 2323, showing few nuclei and few fat globules. *B* = blood vessel. × 1,000.

If the difference in the cells of the interstitial spaces is in the shape of the nuclei, and the shape of the nuclei is merely due to mechanical causes, can we call any of these cells interstitial cells with the accepted meaning of the word? We could form a complete series of nuclei ranging gradually from the typical elliptical connective tissue nuclei to the round undifferentiated nuclei (Figs. 2, 5, 6). From the figures and statements in Mazzetti's paper, it appears that he has made the shape of the nucleus the important factor in deciding which cells are interstitial cells. But his results, in entire agreement with those of the present paper, show a complete series of gradations from his so-called interstitial cells with round nuclei to typical connective tissue cells. Also he finds a variation in the proportion of interstitial cells in animals of widely different species. Here in the chicken there is as much variation in this regard in birds of the same breed, if we take the shape of the nuclei as the basis for deciding which are interstitial cells, as Mazzetti describes for different genera or orders.

The only other difference evident in the cells of the interstitial tissue is the staining capacity of the nuclei. This difference is not noticeable in just-hatched chicks, but very apparent in the older birds. Some stain very dark, and some remain almost unstained. Table I. gives the mean number and Table II. the percentage of dark and light cells. In ♂s 666, and 1271, there are 12 per cent. and 4 per cent. dark cells, and in ♂s 2323 and 147, there are 11 per cent. and 1 per cent. That would be a great variation in the per cent. of interstitial cells, if a dark staining nucleus could be regarded as a distinctive character of an interstitial cell. But it cannot. I made a differential count of the long and round nuclei among the dark staining ones, and found

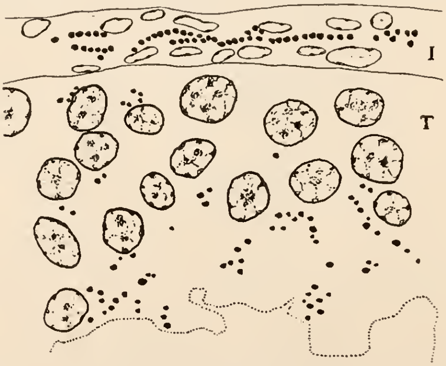

FIG. 7. Section of testis in ♂ 666, showing part of one seminal tubule (*T*) and the adjacent interstitial tissue (*I*). The fat is indicated by black dots. × 1,000.

37 per cent., 13 per cent., 31 per cent. and 54 per cent. of the dark-staining nuclei which have the long shape of typical connective tissue nuclei. It seems then that staining capacity of nuclei is not a satisfactory method of distinguishing interstitial cells.

There is one more point in the frequent descriptions of interstitial cells to be considered. Is there any evidence that the cells of the interstitial tissue are secreting fat? We have already found fat present in testes of just-hatched chicks, but decided that it probably was not a secretion. Fat is also present in the older testes, and is represented by the black dots in the drawings. Comparison of Figs. 5 and 7 with Figs. 6 and 8 will show that the fat is inside of the tubules as well as in the interstitial tissue. The fat globules in the interstitial tissue are not arranged like secretory granules in the cytoplasm of cells. They are mostly in long rows as though packed in spaces between connective tissue fibers. Figs. 5 and 7 show nearly as much fat near typical connective tissue nuclei as near the round or dark-stained nuclei of the "interstitial cells." A comparison of Figs. 7 and 8 shows much fat in Fig. 7 where there are only connective tissue nuclei in the interstitial space, and no fat in Fig. 8, where there is a small group of round dark-staining nuclei. Fig. 9 shows a part of a large mass of interstitial tissue where the nuclei are mostly round. This is evidently a so-called interstitial gland.

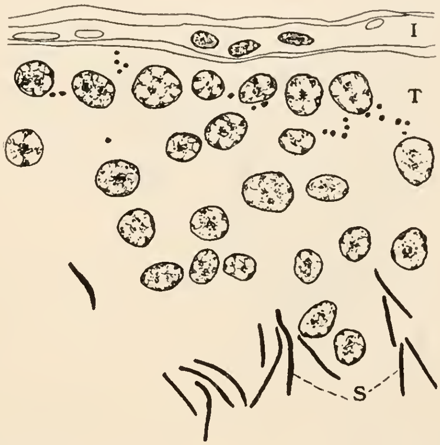

FIG. 8. Same as Fig. 7, but from ♂ 2323. *S* = spermatozoa. × 1,000.

If interstitial cells or cells of interstitial connective tissue are secreting the fat found in the testis we certainly would expect to find it abundant in such an "interstitial gland," or if we prefer not to use that term, in so large a mass of interstitial tissue. However, there is not so much fat as in some of the smaller triangles and narrow spaces. This study shows *no evidence that the fat in the active testis is formed by the interstitial cells.*

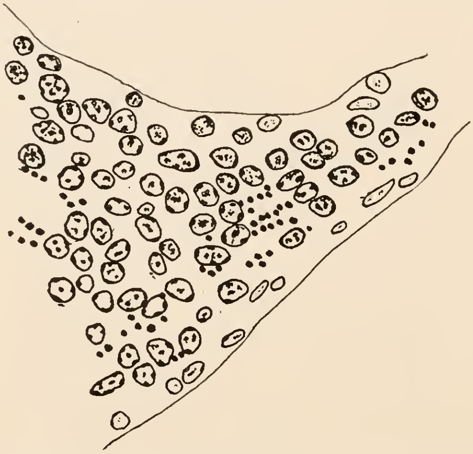

FIG. 9. Part of an " interstitial gland " in ♂ 666, showing many round nuclei, but comparatively little fat. × 1,000.

It seems likely that this fat is being brought to the testis by the general metabolic processes possibly in connection with sexual activity, just as fat is deposited in the yolk of eggs in the hen. This would fit in with the former suggestion that possibly the testis in ♂ 666 is not as far developed as 147 and 2323. The tubules in 666 are smaller and the quantity of fat is greater. It may be in the process of active growth, while 2323 has reached the climax of growth of tubules, and used up most of the fat for this growth.

SUMMARY.

In conclusion, then, I find no cells in the interstitial tissue in the young or old chicken testis with the cell bodies differentiated from the connective tissue fibers. No evidence has been found that differences in shape of the nuclei are indicative of functional differences in the various cells of the interstitial tissue. On the contrary, it appears that these differences in shape depend on mechanical pressure conditions. The difference in the staining capacity of the nuclei is not a basis for cell classification, for the nuclei which take a deep stain include those of all shapes. The fat in the testes is probably not formed by the interstitial tissue at all, but is brought there by the circulation and deposited. So we are brought to the conclusion that there are no "interstitial cells" in the testis of the domestic chicken in the sense that this term has been previously used. Furthermore no evidence has been found in this study which would indicate that an internal secretion of any kind is formed by any cells of the interstitial tissue. This result would appear to derive interest and significance in consideration of the fact that in few animals is there so extensive a development of secondary sexual characters as in the male of the domestic fowl.

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REGENERATION AND REGULATION IN PARAMECIUM CAUDATUM.

FLORENCE PEEBLES, PH.D.

The investigations described here were begun in February, 1910, upon some pure lines supplied through the courtesy of Professor Jennings. The experiments were undertaken with the hope of determining how new races arise, and if possible, to produce by artificial means, a new race differing in size and perhaps in other characteristics from the parent stock.

A long series of experiments was carried on, testing the effect of various foods, and also subjecting the organisms to other changes in the environment. The conjugation of a large and a small individual was followed in order to see if the size of the ex-conjugants was modified by the inequality of the gametes. It was found impossible by any of these methods to produce a race with new characteristics.

In the winter of 1910–1911 a study of the regenerative and regulative power of single individuals and of conjugating pairs was undertaken. It was hoped that after the removal of part of the cytoplasm, and possibly some of the nuclear material as well, a small race might be produced. It soon became evident that the removal of the nuclear material was not possible, for no cell or fragment of a cell (unless cut while in the process of division) recovered after the nucleus was injured. Owing to the extremely small number of successful operations, and the alluring interest of various side issues which arose in connection with the work the main problem was neglected for a time and other experiments, which will be described in this paper, have been carried on at intervals up to the present date.

In November, 1910, in a paper read before the Cambridge Philosophical Society, Levin ('10) described very briefly some experiments on *Paramecium* in which he produced races without a micronucleus, and others where he found it possible to divide a living cell so that each fragment received a portion of the meganucleus. The brevity of the paper makes it difficult to determine how this result was brought about. I have not been able to find a more complete account of these preliminary experiments.

In June, 1911, while working in Professor Boveri's laboratory in Würzburg, I made a series of experiments on large races of *Paramecia* from Munich and Würzburg, and up to May, 1912, these pure lines were used for comparison with material collected in Bryn Mawr. During the course of my investigations a paper appeared by Calkins ('11) in which he describes in detail the behavior of fragments of *Paramecia* after the removal of some of the cytoplasm. My results confirm those of Calkins so exactly that I shall omit a full description of the individual experiments merely giving a summary of the results in tabulated form.

It gives me pleasure to have this opportunity to express my gratitude to Professor Boveri for the many courtesies extended to me during my stay in Würzburg, and to Professor Jennings for his kindness in supplying me with some of his pure lines.

METHODS.

The methods followed were practically those used by Calkins except that instead of treating the cells with neutral red before cutting them, the animals were quieted by placing them in a small drop of tap water thickened with quince seed. Immediately after the operation a few drops of culture fluid were added. All cultures were kept in hollow slides in a moist chamber. The culture medium was changed every twenty-four to forty-eight hours as conditions required. The infusion made from Timothy heads, according to Jennings' method, was the culture fluid most generally employed. A most satisfactory culture medium if rightly used is a .2 per cent. solution of Horlick's Malted Milk.

EXPERIMENTS.

A. *The Behavior of Cells after Removal of Part of the Cytoplasm.*

Thousands of *Paramecia* were cut during the course of these experiments. The fragments were examined four hours after the operation and then again on the following day. All fragments living less than twenty-four hours after the operation were omitted from the records. By this process of elimination the making of tables was greatly simplified.

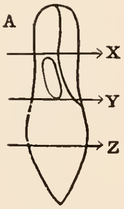


Fig. 1. Diagram of vegetative cell showing regions of the cut for removing the anterior or posterior end and dividing the cell in half.

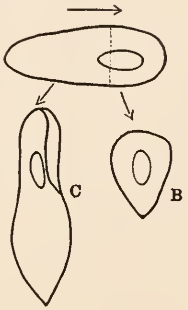


Fig. 2. Fragment of cell from which the anterior end was removed. B and C, the cells formed from it by division in the original plane.

1. *Removal of the Anterior End.*—In this experiment the anterior end of the cell was cut off at the level indicated in Fig. 1, *X*, thus removing from one fourth to one third of the cytoplasm. The small non-nucleated piece (Fig. 1, *A*) anterior to the cut usually disintegrates immediately after the operation, but in a few cases it closed in, forming a spherical mass which swam about rapidly for a day and then died. Such pieces never divide or show any sign of growth. The removal of the end often produces great disturbance in the larger nucleated piece. This is probably due to the fact that the macronucleus lies so near the level of the cut, and the oral groove prevents the closure of the wound. The following table gives the results from eighty-three individuals.

Table I.

Number of Paramecia.	Regeneration Followed by Normal Fission.	Division in Original Plane.	No Division.	Monsters.	Killed.
83	28	42	8	4	2

Thirty-four per cent. of the pieces developed new cilia at the cut end and grew to the normal size before dividing, but nearly fifty per cent. divided in the original plane (represented in all figures by the dotted line) forming a small irregular anterior cell (Fig. 2, *B*) and a normal posterior cell (*C*). The smaller cell may grow rapidly to the normal size and divide into two equal cells, or it may form another small anterior and large posterior cell. At times the anterior cell finally produces a monster.

2. *Removal of the Posterior End.*—The removal of the posterior end of the cell through the region back of the mouth (Fig. 1, *Z*) produces less disturbance than cutting off the anterior end. This result would be expected as there is little danger of injury to the nucleus which lies in front of the cut. The results from this experiment are given in the next table.

Table II.

Number of Paramecia.	Regeneration Followed by Normal Fission.	Division in Original Plane.	No Division.	Monsters.	No Regeneration.
88	55	18	14	1	1

The percentage of fragments which regenerated and divided in the normal manner is much larger than that of Table I. Here sixty-two per cent. after recovery from the operation divided normally, forming a race of the original size, while only thirty-four per cent. of the cells in Table I. regenerated the lost end before dividing. In twenty per cent. of the fragments the division was irregular, resulting in a large anterior and a small posterior cell. This result stands out in sharp contrast to the fifty per cent. dividing irregularly in Table I. The probable explanation of this result is to be found in the fact that when the anterior end is removed the nucleus is either exposed directly to the surrounding medium, or is separated from it by a thin film of cytoplasm, and may be thus stimulated to divide in the plane already laid down before a re-arrangement of symmetry is effected. The result is that one daughter cell contains twice as much cytoplasm as the other, while both have the same amount of nuclear material. When the posterior end is removed (Fig. 1, *Z*) there is an equal amount of cytoplasm back and front of the nucleus, so that a new division plane forms, and when fission takes place each half gets an equal amount of cytoplasm. The later history of the small cells formed by irregular division will, I think, support this suggestion.

B. *The Behavior of Cells Cut in Half Transversely.*

It is extremely difficult to cut a *Paramecium* in half without permanently injuring both halves. The macronucleus almost invariably slips out and the pieces disintegrate within an hour or two. If, however, the nucleus is pushed forward or backward, as the case may be, into one of the halves that piece lives and will finally divide into two normal individuals. Out of forty cells thirty died without fission, and ten produced new races of the normal size.

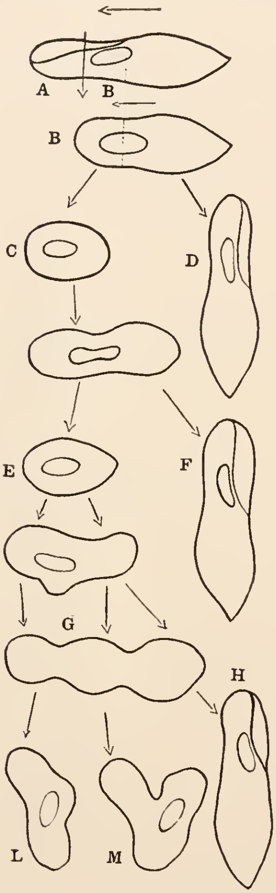


Fig. 3. Diagram giving the history of the formation of three normal races and two monsters from a cell from which the anterior end was cut off.

All of the experiments described so far were made, without exception, upon cells in the vegetative condition. None of the cells had recently divided or were preparing to divide.

C. *The Formation of Monsters.*

Calkins ('11) has shown that it is possible to produce from a small fragment a mass of such dimensions and structure that it represents many individuals, having several mouths, oral grooves, and vacuoles. I have not succeeded in obtaining more than a dozen of these monsters in the course of my investigations. Most of them developed during the warm weather in the latter part of June. Thinking that the temperature had something to do with their appearance I began a series of experiments under various degrees of warmth above that of the laboratory, but did not succeed in producing any more monsters than at room temperature. A brief description of several of these monsters may throw some light on the question of their mode of origin.

No. 1.—This monster arose from one of the fragments in Table I. where the anterior end of the cell was removed (Fig. 3). At the end of twenty-four hours the fragment (*B*) appeared active but showed no sign of fission; the cut end was slightly rounded. Before the end of forty-eight hours division took place in the original plane producing a small individual (*C*) and a large one (*D*). On the following day *C* began to divide. During the next night fission was completed and another small (*E*) and large (*F*) cell formed. On the fourth day *E* had increased in size, and showed signs of division. On the fifth day it had formed a chain of three cells (*G*), the most posterior of which formed a normal individual. The chain continued to throw off normal cells posteriorly until finally after a period of growth a mass was left which separated into two parts (*L* and *M*). These when killed and stained were found to possess several mouths, but neither piece contained more than one nucleus.

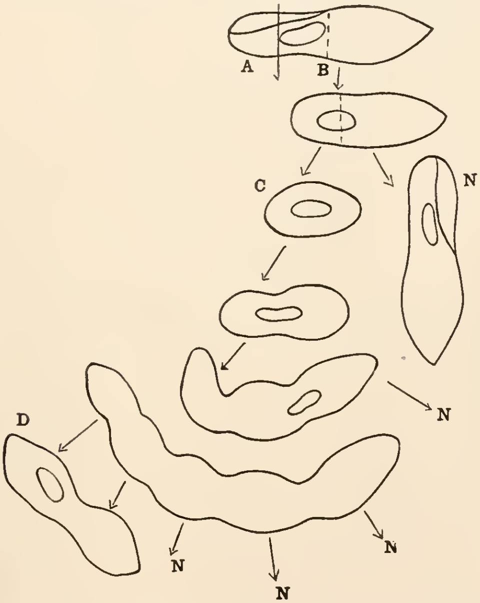


FIG. 4. Diagram showing the formation of five normal races and one monster.

No. 2.—The second monster arose in the same way from a small anterior cell produced by the division of a fragment in the original plane. In this case a chain of five parts (Fig. 4) formed from the small piece (*C*). Finally the chain divided posteriorly into a series of normal individuals (*N*) leaving anteriorly one double cell (*D*). On the seventh day this monster was killed and stained. Three mouths, two vacuoles, and only one nucleus were present.

No. 3.—The third monster (Fig. 5) formed directly from the small anterior piece (*C*) without dividing. The piece grew rapidly for three days developing processes resembling budding individuals but none were thrown off. At the end of the third day the mass (*m*) was killed. Again only one nucleus was found but three mouths and four vacuoles were present.

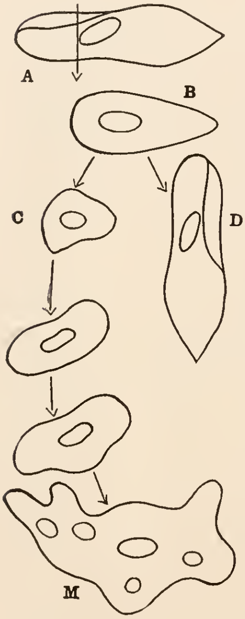


Fig. 5. Diagram showing the formation of a monster from a small cell without division.

Such cases as these demonstrate that there is great disturbance produced through the removal of some of the cytoplasm of a vegetative cell. The small piece (Fig. 5, *C*) contained all of the nuclear material for a normal cell, but less than half of the protoplasm required. Before the cell-body has attained its full growth the nucleus is ready to divide, and in turn when the cytoplasm is ready to divide the nucleus is not. This condition of affairs produces a complete loss of balance in the mechanism of division and the result is that irregular cells and monsters are formed and the cell rarely ever regains its normal condition.

D. *The Effect upon Cells Cut During Division.*

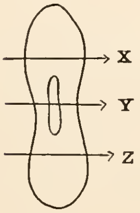
For these experiments *Paramecia* were selected which were in the early stages of fission, at a time when the nucleus was elongated and the body slightly constricted (Fig. 6).

Experiment 1.—When the cell is cut in half through the plane of division (Fig. 6, *Y*) both cytoplasm and nucleus are divided equally. The experiment is a comparatively simple one and is usually successful, both halves living and forming normal races.

The survival of both pieces depends largely on the stage of fission reached at the time of the operation. The nearer the process is to completion the more likely is the recovery of both halves.

Experiment 2.—When the cut was made through the center of the anterior or the posterior half of the dividing cell (Fig. 6, *X* and *Z*) the small non-nucleated piece always died, and the large piece continued to divide in the original plane forming a small cell (Fig. 7, *C*) and a large cell (*D*).

Experiment 3.—When the cut is made in either half of the dividing cell near the plane of division (Fig. 8, *X* and *Y*) the small fragment (Fig. 9, *B*) remains attached to the other half, and is gradually absorbed. In a few cases division continued in the original plane, but this occurred only in those cells where fission was almost completed at the time of the operation.



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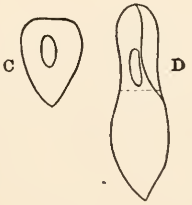

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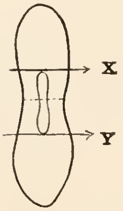

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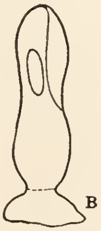

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Fig. 6. Dividing cell showing the region of the cuts.

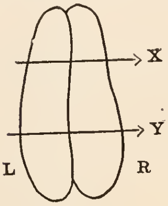
Fig. 7. Small and large individual formed by division in the original plane after removal of the anterior end of a cell during the process of fission.

Fig. 8. Diagram showing cuts made near the center of a dividing cell.

Fig. 9. Cell with fragment of the posterior half still attached.

E. *The Effect of Cutting Cells During Conjugation.*

During epidemics of conjugation pairs which had become firmly united were isolated and from these either the anterior or the posterior ends were removed (Fig. 10, *X* and *Y*). None of the pairs were cut through the center. The non-nucleated fragments always died shortly after the operation, while the larger ones either remained in contact for several hours or separated at once. Regeneration in these pieces is exceedingly slow and fission is greatly delayed. Truncated fragments (Fig. 11, *L* and *R*) have been observed for days after the operation, swimming about rapidly but showing no sign of fission, or of regeneration of the lost end. Although the conjugating pairs were cut at the same level after separation one fragment was almost always smaller than the other (Fig. 11). The small one (*R*) generally dies, and the large one (*L*) divides to form a normal race, when both halves lived the smaller one finally divided and after many generations the descendants regained the normal size. The fission of all fragments of conjugating pairs was regular; I have never seen a large and a small cell formed by division in the original plane as so frequently happens when cells are cut in the vegetative condition. This regularity would indicate that the division plane is lost during conjugation, and that when it is re-formed it is laid down in the center of the fragment.



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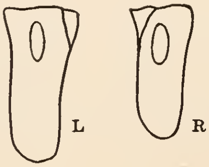

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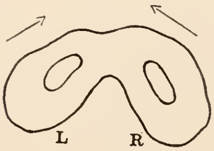

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FIG. 10. Conjugating cells showing region of the cut.

FIG. 11. The right and left halves of a conjugating pair after removal of the anterior end.

FIG. 12. Left and right halves of a conjugating pair grafted together.

In three of the operations the halves were pressed together so firmly by the edge of the knife that the fragments grew together at the cut surfaces. These grafted fragments (Fig. 12) showed great activity for a few days, but finally died without dividing. One of these was stained to see the nuclear condition. There was a large single nucleus in each half. If these grafts could be kept and fission induced, the regulation would, no doubt, be of value in throwing light on the problem of the "nucleus-protoplasm-relation."

F. *The Effect of Cutting Cells Shortly after Fission.*

In order to obtain cells at a definite period in their development they were removed from the stock culture, and as soon as they had separated each cell was isolated in a drop of the cuture fluid. A period of two to five hours was allowed for growth, then the cells were cut and the further development of the nucleated fragments observed. The power of regeneration is present in these cells, but very few recover from the operation. In the actively growing cell the cytoplasm is in a less viscid state than it is in the vegetative cells, and it is for this reason that injury to the ectosarc results, in ninety per cent. of the operations, in the escape of the entire contents of the cell. In successful operations removal of the anterior or the posterior end give the same general results as in the vegetative cells. The most striking fact brought out is that as early as two and a half hours after fission the next division plane is determined. A brief description of a few experiments will demonstrate this.

R. 28. Two sister cells were isolated two and a half hours after fission; the anterior end of one and the posterior end of the other were removed (Fig. 13, *A* and *P*). On the following day the nucleated fragments were active but had not yet divided. By noon of the same day *A* had formed two normal cells and *P* had divided into a small and a large cell. Later the smaller cells grew to the normal size; they were then killed.

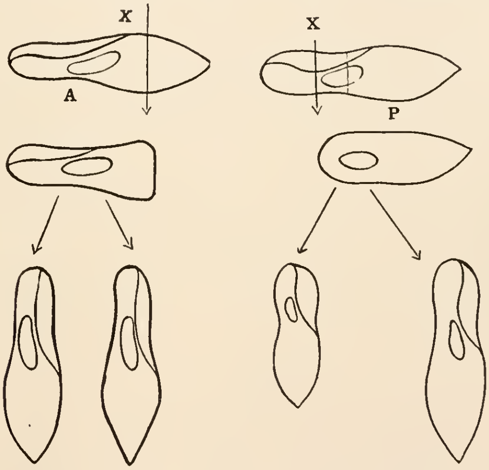

FIG. 13. Two sister cells cut at the same time, showing the formation of a normal race from the one from which the posterior end was removed, and the irregular cells formed from the cell from which the anterior end was removed.

R. 59. The anterior end of a cell was cut off four hours after fission. The nucleated fragment divided irregularly into a small anterior and a large posterior cell.

R. 30. One monster was obtained from a cell cut five hours after fission. Almost the entire anterior half was removed (Fig. 14, *X*). The wound closed in very slowly, the fragment increased in size (*C*), and not until the fourth day after the operation was any sign of division observed; then a constriction appeared near the anterior end which finally assumed the shape of an individual (Fig. 14, *D*). On the tenth day the mass divided into two irregular cells (*E* and *F*) each containing only one nucleus. It is evident that fission here was not only delayed but the whole mechanism of cell division was thrown out of order. The disturbance here was greater than when the cut was made further forward, and may have been due to injury to the nucleus.

In these young cells there was no sign of an increased rate of fission after removal of a part of the cytoplasm such as was observed in vegetative cells where three or four divisions followed in quick succession in the first twenty-four hours after the operation.

G. *A Comparison of the Behavior of the Nucleated Fragments from Four Different Races.*

In order to compare the power of regeneration in different races four distinct lines were selected and the following table compiled.

Table III.

Race.	Food.	Regeneration Followed by Normal Division.		Division in Original Plane.		No Regeneration.	
		Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.	Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.	Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.
C	M. Milk	67	100	33	9	0	0
M	M. Milk	23	25	59	30	18	45
E	Timothy	50	88	50	12	0	0
S	Timothy	25.5	43	49	30	25.5	27

Such a table as this demonstrates clearly that the power of regeneration varies greatly in different races. It also shows that in all four races there is greater disturbance when the anterior end is removed than there is when the posterior end is cut off. This table does not show the tremendous differences in the behavior which result from the condition of individual cells at the time of the operation. There is a definite correlation between the behavior of fragments and the periods of depression to which cultures are subject. At such times a race which normally shows great power of regeneration fails both to regenerate and to divide. The power to regenerate is not so much a characteristic of a race as it is an indication of the vitality of the individual cell. *Paramecium* taken from a pure line will regenerate in ninety cases out of a hundred if the cytoplasm is in a viscid state and the animals are well fed. When the cells are starved or in a period of depression from other causes, the rate of division is slow and the power of regeneration is greatly reduced or altogether lost.

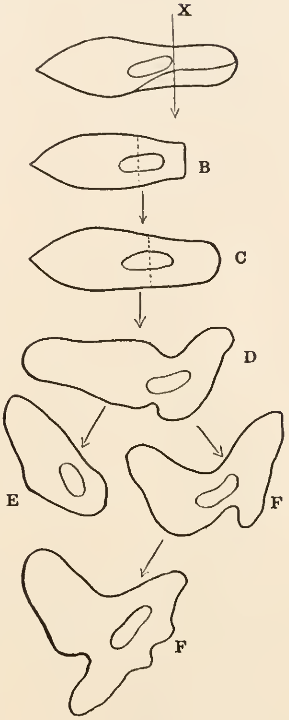

FIG. 14. Diagram giving the history of a fragment cut close to the nucleus.

H. *Can the Size of a Race be Reduced by Removal of Part of the Cytoplasm?*

In his extensive and careful investigations on *Paramecium* Jennings ('10) has shown that race size is inherited and that a new race of a different size does not arise within a pure line. Popoff ('09) claims that it is possible through experimental means to change the size of the race and to maintain the altered cell size for an indefinite period. The forms upon which Popoff made his observations were *Frontonia leucas* and *Stentor cœruleus.*

In order to determine whether or not a loss of cytoplasm alone would reduce the size of a race permanently I undertook a long series of experiments on certain pure lines where the average size was definitely ascertained before the operation was performed, and the average length and breadth of all cells arising from small fragments was compared. If these measurements had been made from cells of the first few generations arising from such fragments the conclusion would have been reached that it is not only possible, but quite a simple matter to produce a small race from a large one. It is necessary to keep such cultures for many generations until after repeated divisions the normal size is regained. The method followed in these experiments may be illustrated by giving one example. All measurements were made with a micrometer under a Zeiss objective 3, ocular 1, after the cells were killed in Worcester's fluid.

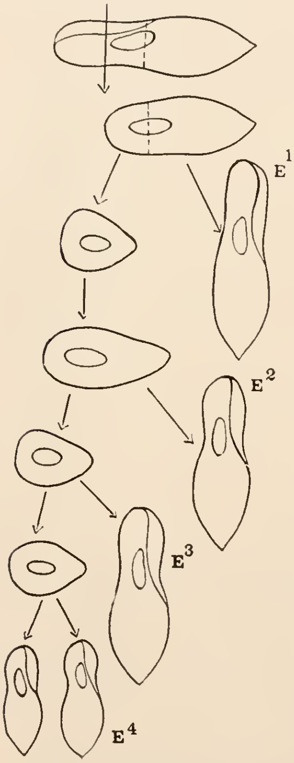

Fig. 15. Diagram showing the formation of normal races after irregular division

Experiment E.—The anterior end of the cell was removed (Fig. 15), and as soon as the nucleated fragment divided into a small and a large cell the two individuals were separated. The large piece continued to divide forming a normal race which we shall distinguish as E^1. The small piece after a period of growth divided irregularly into a small anterior and a larger posterior cell. The descendants of the posterior cell formed a race which is designated as E^2. The next division of the small piece was again irregular, the posterior cell exceeding the anterior in size. The race formed from this large cell was designated E^3. The small anterior piece at the next division formed two cells of equal size; these were called E^4. There were then four races E^1, E^2, E^3 and E^4 to be compared with the pure line E from which they arose. Careful measurements were made from time to time and during the course of the experiments, and all of the races were kept under the same conditions as the control. The experiment was begun on March 14, and the first measurements made on the twenty-third showed the same average size in E, E^1 and E^2, but E^3 and E^4 were much smaller. The next measurements made on the twenty-sixth showed that E^3 had reached the average size, and a week later the small cells of E^4 were fully as large as the control race.

It is undoubtedly true that a large number of these small fragments, and most of the cells thrown off from the monsters, show signs of extreme feebleness and often die after one or two divisions. It is only those that formed races that are taken into consideration. Small fragments if possessing enough vitality to continue the race will sooner or later become as large as the parent stock. At the present time I have a race descended from a small anterior fragment formed by irregular division. The average size of these cells is slightly larger than that of the pure line from which they arose.

In this connection it is also interesting to note that after removal of the anterior ends of conjugating cells (Fig. 10, *X*) when for some reason the fragments differ in size (Fig. 11, *L* and *R*) the descendants of both halves finally regain the same average size.

GENERAL.

In considering the phenomena of regeneration and regulation in a unicellular organism one must take into account the fact that we have, in such a form as *Paramecium*, a highly differentiated structure. This differentiation is greater in the anterior end of the cell and diminishes posteriorly. Therefore it is not surprising that less disturbance results from removal of the posterior than of the anterior end. Regeneration follows the removal of the posterior end (Fig. 1, *Z*) in most cases, but this does not mean that the entire end is replaced before fission occurs Such fragments divide before the full size is regained, therefore regeneration amounts to nothing more than the healing over of the wound and the formation of new cilia; the regaining of the original body form is brought about by a gradual process of regulation through growth in volume. After the wound is healed a new plane of division is laid down, and through fission two individuals of equal size are produced; when the new plane is not formed division takes place in the original plane and two cells of unlike size are produced. What causes this difference? Why does a fragment usually divide into two cells of equal size when the posterior end is cut off and of unequal size when the anterior end is removed? I have suggested that this is due to the position of the nucleus. When the cut is made close to the anterior end of the nucleus it is stimulated to divide at once while there is no such stimulation when the cut is made through the end of the body at some distance from the posterior end of the nucleus. Popoff claims that the volume of the cytoplasm can be regulated so that it accommodates itself to the volume of the nucleus. In this instance, however, it does not seem to be a question of balance between nuclear volume and cell volume. After removal of one end the fragment contains a whole nucleus and only two thirds to three fourths the normal volume of cytoplasm. After the first irregular division the small piece contains one half of the original nucleus and from one fourth to one third of the cytoplasm. Before the balance is established the cell may divide again into a small and a larger cell, or into two cells of the same size. When cut in half in the vegetative state the surviving fragment contains all of the nucleus and one half of the cytoplasm, yet these cell fragments divide in the normal manner.

It appears then that the ratio of the volume of the cytoplasm to the volume of the nucleus does not explain these results, and we must, therefore, look to some other source for light on this subject. If, as it appears, the division planes are laid down shortly after fission is it not possible that irregularities might result from the disturbance of these planes? I do not believe that there is any visible plane present, but there is strong indication of some such differentiation. If we consider a mature *Paramecium* as possessing a plane through the center as indicated by the double row of dots in Fig. 16 and two potential planes in the middle of each half, at the single row of dots, there are four normal individuals present in one cell. A cut made through the center should be followed by normal division of the surviving half, and this is the case. If the cut is made through either of the other planes we should expect division in the original plane resulting in a small and a large individual, but if the cut were made in front of the most anterior plane or back of the most posterior one there would be two opposing division areas in the same fragment and a reorganization would be necessary; thus both planes would disappear and a new one would form which would bring about symmetrical division. The results of the experiments that I have made seem to support this theory, but in order to test the validity of the suggestion it would be necessary to make careful measurements of each cell used, and it would scarcely be possible to determine with exactness whether or not the cut were made through the region of a division plane or on one side of it.

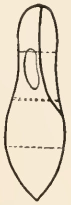

FIG. 16. Diagram of mature cell showing regions of division planes.

The fact that a loss of cytoplasm from such a highly differentiated organism as *Paramecium* does not affect the size of the race is one more proof of the necessity of seeking some way of reaching the nucleus, for if some of the nuclear material could be removed the result might be very different. So far I have found it impossible to keep a fragment alive after injury to the nucleus. It is possible that after repeated removal of cytoplasm from the same cell a small race size could be established, but the difficulties of the operation and the great disturbance produced by it in the cell would render the experiment practically impossible.

SUMMARY.

1. When the anterior end of the cell was removed by a transverse cut through the peristome just in front of the macronucleus, thirty-four per cent. of the fragments regenerated the lost end and divided normally. When the posterior end was removed by a similar cut, back of the mouth, sixty-two per cent. of the nucleated fragments regenerated the lost end and formed a normal race.

2. Removal of the anterior end resulted in irregular division

in fifty per cent. of the nucleated fragments, while removal of the posterior end caused irregular division in only twenty per cent. of the pieces.

3. *Paramecia* cut in half transversely usually die as the nucleus is forced out by the pressure of the knife. When the nucleus remains in one of the halves regeneration is followed in that half by normal division.

4. Monsters develop from fragments composed of more than half of the protoplasm of a normal *Paramecium*, but never develop when a cell is cut exactly in half.

5. A fixed division plane is present in the normal cell as early as two and a half hours after fission. It is possible in vegetative cells, where fission has been delayed for a period, that more than one plane is laid down. This would account for the fact that after cutting off one end of the cell several divisions follow in quick succession.

6. The power of regeneration varies in different races, and in different individuals of the same race. It is the index of the condition of the cytoplasm.

7. The removal of a portion of the cytoplasm does not result in the production of smaller individuals. After several generations have been produced the normal size is regained.

Bryn Mawr, Pa.,
May 11, 1912.

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ON THE RECOGNITION OF SEX THROUGH EXTERNAL CHARACTERS IN THE YOUNG RAT.

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The rat is an animal widely used for scientific investigation. Especially through the work on growth by Donaldson and his associates, it has in some respects been more carefully studied than any other animal. The recognition of sex from external characters in the young living rats is therefore sometimes a matter of considerable importance in selecting animals for various lines of investigation.

That considerable inconvenience has been occasioned by difficulty in the early recognition of sex in the living rat is evident. King,[1] for example, states (p. 385) that " The sex of a newborn rat cannot be ascertained with any degree of certainty unless the animal is killed and dissected. When the rats are 14–16 days old, however, the sexes are easily distinguished as Dr. Stotsenberg has discovered, since the mammæ are clearly visible at this time. After this period the hair covers the entire body and it becomes very difficult to distinguish the sexes in the living young until they are several weeks old." Slonaker[2] similarly notes (p. 4) that " Owing to the fact that it is difficult to determine the sex of the young rats with accuracy the sexes were not distributed as I would have wished."

Since no method of distinguishing the sexes in the young rat (except that noted by King) has, so far as I know, been published, the following observations, which were made during a study of growth in the white rat, may be of some interest and value. While the observations were made exclusively upon the white or albino rat (*Mus norvegicus albinus*), they will doubtless apply also to the ordinary gray or brown rat (*Mus norvegicus*). A few observations upon young gray mice indicate that (at least with respect to the ano-genital distance and the size of the genital papilla) the same method may be utilized in distinguishing the sexes in this and perhaps other members of the genus.

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[1] Helen Dean King, "The Effects of Semi-Spaying and of Semi-Castration on the Sex Ratio of the Albino Rat (*Mus Norvegicus albinus*)." *Journal of Experimental Zoölogy*, Vol. 10, No. 4, 1911.

[2] James Rollin Slonaker, "The Effect of a Strictly Vegetable Diet on the Spontaneous Activity, the Rate of Growth, and the Longevity of the Albino Rat." Leland Stanford Junior University Publications. University Series, April 2, 1912.

Table I.

Ano-genital Distance in Young Albino Rats of Various Ages.

Age.	Number of Each Sex.		Average Gross Body Weight (and Range).		Average Ano-genital Distance (and Range).	
	Male.	Female.	Male, Grams.	Female, Grams.	Male, Mm.	Female, Mm.
Newborn.	10	12	5.7 (5.1–6.3)	5.4 (4.7–6.3)	2.8 (2.5–3.0)	1.2 (1.0–1.5)
7 days . .	17	26	11.0 (8.8–12.9)	10.4 (6.7–12.2)	5.2 (4.5–6.0)	2.7 (2.0–3.5)
14 days . .	13	15	19.5 (17.0–21.9)	18.2 (15.3–19.9)	8.2 (7.0–9.0)	4.9 (4.0–6.0)
20 days . .	19	26	27.4 (15.6–40.0)	27.4 (19.7–41.6)	12 (10–14)	7 (6–9)
6–7 weeks	19	13	73.3 (57–114)	71 (23.5–95)	21 (17–25)	13 (9–15)

The observations may be grouped under four headings. The first and most important character to be noted is the *ano-genital distance*, which is the distance measured from the anal aperture to the base of the genital papilla (clitoris or penis). The measurements are summarized in Table I. They were made on the living animals at the various ages from birth to 6 or 7 weeks, when the sexes are easily distinguished by the ordinary characters. It will be observed that *in all cases the ano-genital distance in the male is much greater than in the female of the same age.* It averages nearly twice as great. Although, owing to the great range of variation in the size of the body, the largest distance recorded in the table for the female sometimes approaches the smallest found in the male for the same age, there is never *in any given litter* the slightest difficulty by this measurement in determining the sex with ease and accuracy.

A second characteristic is found in the *size of the genital papilla.* This (anlage of penis or clitoris) is always larger and more prominent in the male than in the female. This difference is clearly evident when the sexes are compared in individuals of the same litter.

A third characteristic is the *presence of the mammæ*, already mentioned by King as observed by Stotsenberg. I have confirmed the statement that the mammæ (nipples) are readily visible in the female of 14–15 days, and that after that time they become hidden by the hair. During lactation, of course, they again appear. I have also observed further that the nipples are visible at an earlier age than that mentioned. During the entire second week, and at least in some cases during the first week, they can be noted by careful observation in the female. From the time they first appear, the definite number (six pairs) is present.

When the hair coat becomes well developed, at about 16 or 17 days of age (when also the eyes are opened), a fourth sexual characteristic may be noted. In the male, a small area just ventral to the anus remains bare. In later stages it becomes to a certain extent covered with short hairs, but always remains relatively bare. This corresponds to the dorsal part of the scrotal area, as may be noted after the sixth week, when the testes occasionally descend. In the female, there is a corresponding bare, or relatively bare, strip which extends all the way from the anus to the genital papilla (clitoris). The part of this bare strip just dorsal to the clitoris corresponds to the vaginal aperture; but the aperture does not appear until the middle or end of the second month. In one case, a female bore a litter at the age of 10 weeks, and must therefore have become pregnant at about 7 weeks. Lantz[1] cites a case where a white rat is said to have given birth to 11 young when only 8 weeks old, and which therefore must have bred when only 5 weeks old.

It is evident from the foregoing that of the four characteristics noted for distinguishing the sexes in young rats, one (visibility of nipples) applies only before the age of about 16 days; one (bare areas in ano-genital region) applies only after that age; while the other two (ano-genital distance and size of genital papilla) apply to all ages.

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[1] David E. Lantz, Natural History of the Rat. In "The Rat and its Relation to the Public Health," by various authors. P. H. and M. H. Service, Washington, 1910.

THE LINKAGE OF TWO FACTORS IN DROSOPHILA THAT ARE NOT SEX-LINKED.

T. H. MORGAN AND CLARA J. LYNCH.

In addition to the eight sex-linked factors described for *Drosophila*, a number of other factors have been found that are not sex-linked. They are now being studied in order to discover which, if any, of them are linked to each other. Two that have been found to be linked are here described. These are the yellow factor in the absence of which the flies are black, and a factor (in the complex of factors that produces the normal wing) in the absence of which the fly is wingless.

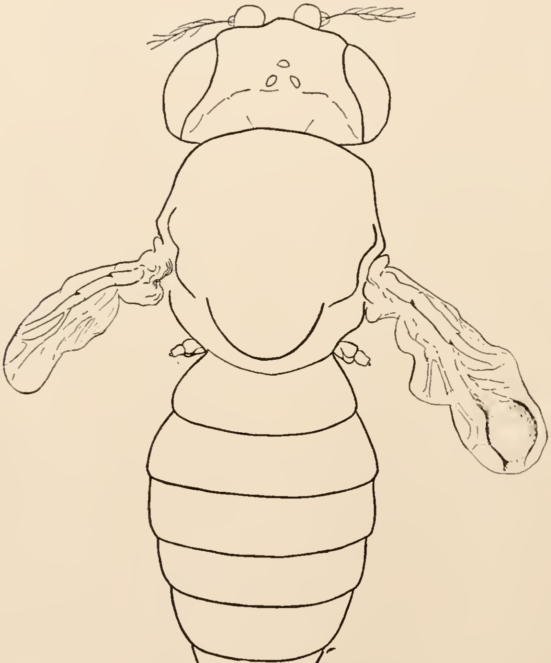

Fig. A.

In previous papers on *Drosophila* a large amount of data has been given showing that in regard to any sex-linked factor, the, yellow factor, *Y*, follows Mendel's law of independent segregation; in other words, the yellow factor segregates independently of all sex-linked factors. The fact that wingless flies do not show sex-linked inheritance has also been reported, but the character of the wing itself and its mode of origin have not been fully described.

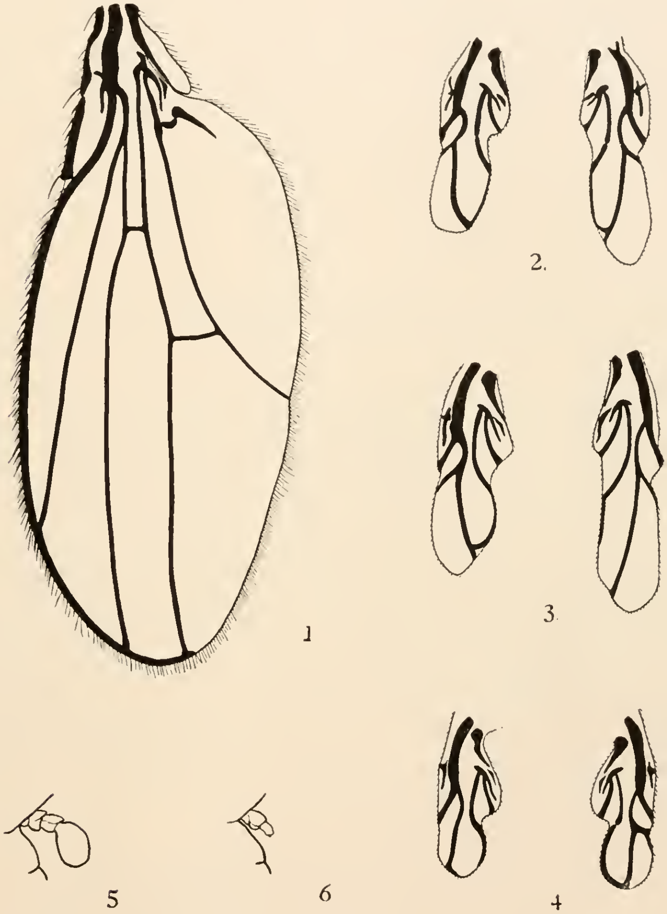

Fig. B.

The size in relation to the body, and general character of the wings are shown in Fig. A. They are little more than scales held out at right angles at the sides of the body. The details of the venation are shown in the three pairs of wings shown in Figs. B, 2, 3 and 4, while for comparison a normal ("long") wing drawn to scale is shown in Fig. 1.

The wingless wing represents in the main the basal portion of the normal wing. The marginal vein is missing, but the cross vein connecting the third and fourth longitudinal veins is often present as an apparently marginal vein. The basal parts of all five longitudinal veins are present.

The balancers are much reduced in size and modified in character. A normal balancer is shown in Fig. 5 and one from a wingless fly in Fig. 6. The terminal segment which is large in the wild fly is represented by only a trace and the second segment is reduced, but the basal segment approaches in size that of the wild type.

The wingless flies appeared in the "truncate" stock and represented at first the wingless condition of the truncate type of wing; owing to the low viability of the truncate flies the winglessness was transferred to normal stock, but as it is difficult or impossible to distinguish between Wingless of truncate stock and wingless of long-winged stock the material was not at first quite homogeneous except in so far as being wingless. There was also present at first in the wingless stock another character, namely, balloon wings, which appeared at about the same time as Wingless in the truncate stock and proved as difficult to separate from the Wingless as Truncate itself. As a result, balloon wings appear in the first cross given below. The cross was made over a year ago by Miss E. M. Wallace.

P_1		F_1		F_2	
				Winged ♀,	1,136
				Winged ♂,	1,038
Wingless ♂	=	Winged ♂	=	Wingless ♀,	259
Winged ♀		Winged ♀		Wingless ♂,	236
				Balloon ♀,	12
				Balloon ♂,	3

The sum of the winged and balloon is 2,189 and that of the wingless is 495, which gives a ratio of 4.42 to 1. It is clear that winglessness is recessive to normal, that it is not sex-linked, and that it has a lower viability than has the normal. The reciprocal cross gave:

P_1
Winged ♂
Wingless ♀
=
F_1
Winged ♀
Winged ♂
=
F_2

F_2	
Winged ♀,	694
Winged ♂,	639
Wingless ♀,	146
Wingless ♂,	127
Balloon ♀,	4
Balloon ♂,	1
Miniature ♀,	1
Miniature ♂,	24

One at least of the grandparental wingless flies must have been responsible for the miniatures, but the numbers are too small to affect the other ratio seriously. There were 1,363 long (including balloon and miniature) flies, and 273 wingless, or 4.9 to 1.

In a recent cross between long-winged ♀ and wingless ♂ there were present in the F_2 generation 1,081 long winged F_2 flies, and 280 Wingless; a ratio of 3.9 to 1. The reciprocal cross gave in F_2 1,213 long-winged flies and 273 Wingless or a ratio of 4.4 to 1. Compared with the preceding the results show that the viability of the Wingless has not changed. It is curious to find that the F_2 ratio in this cross and its reciprocal differ in both instances in the same direction as in the earlier cross, but this may be only a coincidence.

LINKAGE OF THE YELLOW FACTOR, *Y*, AND THE WING FACTOR, *W*.

The linkage in question is most simply shown in the following result: wingless Grays were mated to long-winged Blacks, and gave in F_1 long-winged gray males and females. These inbred gave in the F_2 generation:

Winged Gray.		Winged Black.		Wingless Gray.		Wingless Black.	
♂	♀	♂	♀	♂	♀	♂	♀
854	1,004	410	506	226	329	0	0

There are no wingless black flies in the F_2 generation, which the Mendelian expectation calls for. Their absence can only be explained by strong linkage of the yellow factor and the factor for wings. The Mendelian expectation (unmodified by linkage and viability) is three long Grays to one long Black (3 : 1). *There are actually 1,858 long gray flies to 916 long black, or a ratio of 2 to 1. This is the linkage ratio when two strongly or completely linked factors are concerned.*

Since the males and females count equally in the result it is not necessary to separate them. We are indebted to Mr. A. H. Sturtevant for the following F_2 count derived from the same F_1 material as the last. We are also indebted to him for being first in pointing out the interpretation of this result.

Winged Gray.	Winged Black.	Wingless Gray.	Wingless Black.
458	230	185	0

There are 458 winged Grays to 230 winged Blacks, or almost exactly 2 : 1.

In the following analysis Y = yellow factor; y its absence; B = black factor (sex-linked), and b its absence. The formula for the female gray fly is $YBXYBX$, and for the male, $YBXYb$. The formula for the female black fly is $yBXyBX$, and for the male is $yBXyb$.

The evidence indicates that the wingless condition differs from the winged condition by a loss. Large W may then stand for that "factor" in the original complex that has been lost. In this sense large W means in this case Wings (here long); *and small* w *stands for this loss and in the formulæ represents the wingless condition, which is of course the result of the interaction of all the remaining wing factors.*

	$wYBX$—$wYBX$	Wingless gray ♀.
	$WyBX$—Wyb	Winged black ♂.
F_1	$wYBX$ $WyBX$	Winged gray ♀.
	$wYBX$ Wyb	Winged gray ♂.
Gametes of	$wYBX$—$WyBX$	Eggs.
F_1	$WyBX$—$wYBX$—Wyb—wYb	Sperm.
	$wYBX$ $WyBX$	Winged gray ♀.
	$wYBX$ $wYBX$	Wingless gray ♀.
	$WyBX$ $WyBX$	Winged black ♀.
	$WyBX$ $wYBX$	Winged gray ♀.

wYBX Wyb	Winged gray ♂.
wYBX wYb	Wingless gray ♂.
WyBX Wyb	Winged black ♂.
WyBX wYb	Winged gray ♂.

In the F_1 female, gametes having the composition *WYBX* and *wyBX* would be expected on any assumption except that of strong linkage of *wY* and *Wy*, and likewise in the male, the gametes *WYBX*, *wyBX*, *WYb* and *wyb* would be expected. For the sake of convenience I have omitted to represent the cross-over gametes in the above analysis.

In another cross, miniature-winged, black, white-eyed flies were mated to wingless, gray, red-eyed flies. The result is complex, but only in the sense of giving in one cross 20, F_2, classes, and in the reciprocal cross 13, F_2, classes. The results, however, as far as linkage is concerned, are perfectly evident. The fact is also brought out that the factor for Wings shows no linkage to the sex-linked factor (*C*) for eye color, and to (*R*) the factor in the absence of which the wings are miniature.

Miniature White Bl ♂ by Wingless Red Gray ♀
╲╱
GR Long ♂ and ♀
╲╱
F_2 Females

GRL	*BlRL*	*GR* Wingless
532	268	235

Males

GRL	*GWhL*	*BlRL*	*BlWhL*	*GRM*	*GWhM*	*BlRM*	*BlWhM*	*GRW-g-s*	*GWhW-g-s*
173	49	81	38	92	165	30	52	117	90

There are three classes of females and ten classes of males. The wingless flies only occur as Gray—no black wingless flies being present. The alteration in the ratios, due to the linkage between the two sex-linked factors *C* and *R*, is neglected here. The analysis follows:

	wRMCBYX—wRMCBYX	Wingless red gray ♀.
	WrMcByX—Wrmcby	Min. white black ♂.
F_1	*wRMCBYXWrMcByX*	Winged gray red ♀.
	wRMCBYXWrmcby	Winged gray red ♂.

Gam- *WRMCByX–wRMCBYX–WrMcByX–wrMcBYX* } eggs
etes *WRMcByX–wRMcBYX–WrMCByX–wrMCBYX* }
of F$_1$ *WRMCByX—wRMCBYX—Wrmcby—wrmcbY* Sperm.

Females.

WRMCByXWRMCByX	Long red black.
WRMCByXwRMCBYX	Long red gray.
WRMcByXWRMCByX	Long red black.
WRMcByXwRMCBYX	Long red gray.
wRMCBYXWRMCByX	Long red gray.
wRMCBYXwRMCBYX	Wingless red gray.
wRMcBYXWRMCByX	Long red gray.
wRMcBYXwRMCBYX	Wingless red gray.
WrMcByXWRMCByX	Long red black.
WrMcByXwRMCBYX	Long red gray.
WrMCByXWRMCByX	Long red black.
WrMCByXwRMCBYX	Long red gray.
wrMcBYXWRMCByX	Long red gray.
wrMcBYXwRMCBYX	Wingless red gray.
wrMCBYXWRMCByX	Long red gray.
wrMCBYXwRMCBYX	Wingless red gray.

Males.

WRMCByXWrmcby	Long red black.
WRMCByXwrmcbY	Long red gray.
WRMcByXWrmcby	Long white black.
WRMcByXwrmcbY	Long white gray.
wRMCBYXWrmcby	Long red gray.
wRMCBYXwrmcbY	Wingless red gray.
wRMcBYXWrmcby	Long white gray.
wRMcBYXwrmcbY	Wingless white gray.
WrMcByXWrmcby	Min. white black.
WrMcByXwrmcbY	Min. white gray.
WrMCByXWrmcby	Min. red black.
WrMCByXwrmcbY	Min. red gray.
wrMcBYXWrmcby	Min. white gray.
wrMcBYXwrmcbY	Wingless white gray.
wrMCBYXWrmcby	Min. red gray.
wrMCBYXwrmcbY	Wingless red gray.

The reciprocal cross gave the following results.

Minature White Bl ♀ by Wingless Red Gray ♂

Gray Red ♀ and Miniature Gray White ♂

Females.

GRL	*GWhL*	*BlRL*	*BlWhL*	*GRM*	*GWhM*	*BlRM*	*BlWhM*	*GRW-g-s*	*GWhW-g-s*
220	45	88	45	96	197	46	62	113	95
468				401				208	

Males.

GRL	*GWhL*	*BlRL*	*BlWhL*	*GRM*	*GWhM*	*BlRM*	*BWhM*	*GRW-g-s*	*GWhW-g-s*
225	96	120	35	92	201	41	99	131	105
473				433				236	

In this reciprocal cross there are ten classes each of females and males. Each wingless class is made up (as in the other cross also) of wingless long and wingless miniature. These, if they could be separated, would give, in all, twelve classes of males and females.

The analysis of this cross is similar in principle to that given above and can readily be reproduced by the method used for that cross. How closely the yellow factor and that for Wing are united will be determined later by suitable tests.

On the assumption that factors are contained in chromosomes, it will be apparent that this new case of linkage means that both *W* and *Y* are contained in a chromosome different from the sex chromosome.

After the foregoing results were completed, a doubt arose as to whether we could with certainty detect black wingless flies, even if present, since they would lack one of the most characteristic features of black flies, namely, the wings. We determined to test some of the F_2 flies by mating to long-winged black flies, for we thought, at that time, that this would be a legitimate test for the presence of black flies amongst the wingless offspring. But while it is true that the appearance of black offspring would be expected, if black flies were present, nevertheless the appearance of such flies does not in itself show that black flies were actually present amongst the flies tested; for, as the following analysis will show, crossing over may have occurred when the gametes were produced, and yet no F_2 black wingless flies result. The test showed, in fact, that crossing over had occurred, but the result was obscured, because, necessarily, the F_2 flies would still be gray, unless the crossing over had occurred simultaneously in both sexes and the cross-over gametes happened to meet. The following experiments will illustrate this point:

Seven F_2 wingless males were paired with 15 long-winged black females, and gave 133 gray females, and 96 gray males.

But five wingless females mated to fifteen long black males gave: Gray, ♀, 64; ♂, 61; Black, ♀, 8; ♂, 7. The last result shows that crossing over had occurred in the gametes of one F_1 fly, which gave rise to one fly (at least) that was heterozygous for color, and from this fly came the black flies that appeared in the test. The point of particular interest is that this crossing over had not been apparent in the F_2 gray flies, and the reason for this is not far to seek. If crossing over between wY and Wy in the gametes of the F_1 generation had occurred, the gametes would be: WYB, wYB, WyB, wyB.

If this fly were fertilized by an ordinary F_1 male, whose gametes would be wYB and WyB, all the wingless flies would be gray, and those grays derived from the crossing-over (however rare their occurrence) would be heterozygous for color. Only a back cross by black flies would make evident in the next generation that crossing-over had really occurred. How often it occurs remains to be discovered by further experiments. The ratio of 2 to 1 in the F_2 gray flies shows, however, that crossing-over is not common, and the absence of black wingless flies in the F_2 generation indicates this even better. For, if crossing over were common the cross-over gametes of the F_1 flies would occur often enough for like to meet like and produce black wingless flies.

ON COUPLING OF CERTAIN SEX-LINKED CHARACTERS IN DROSOPHILA.

JOHN S. DEXTER.

In the course of his experiments on the modes of inheritance in *Drosophila*, Morgan has called attention[1] to the fact that when flies bearing two or more pairs of characters of certain sorts are crossed, these characters appear in the F_2 generation coupled as in the original combination (*i. e.*, in the grandparents). This coupling is not complete, but is much larger than is to be expected if the different pairs of characters were independently mendelizing. In a letter to me last autumn he suggested that "in order to find out if there is here some deep-lying principle or only some irregularity, it will be necessary to obtain very large numbers of the F_2's; let us say ten or twenty thousand."

Morgan has offered an explanation of this coupling[2] on the supposition that the factors concerned in the production of the characters lie near together in the chromosomes, and that the twisting of homologous chromosomes about each other in the strepsinema stage of gametogenesis which causes both maternal and paternal characters to lie on each side of the double chromosome, does not separate certain factors that lie very close together, so that when the chromosomes split, these factors will both occupy the same gamete.

It was in order to test these theories that I undertook, at Dr. Morgan's suggestion, to raise "large numbers of the F_2's."

Dr. Morgan's kindly advice and aid have helped me a great deal in this work. I wish also to record here and express my appreciation of the enthusiastic assistance of Miss Margueritte Harmon and Mr. Felix Gustafson, without whose aid the work could not have been done at this time.

I began with pure stock of *Drosophila* of two kinds: Flies with normal body color and red eyes (this is the normal wild fly), and flies with a yellow body and white eyes; both stocks were sent to me by Morgan. The origin of these mutations, and the facts of their individual inheritance, have been described by Morgan[1] in earlier papers.

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[1] *Jour. Exp. Zoöl.*, Vol. 11, No. 4, Nov., 1911, p. 393.

[2] *Science*, N.S., Vol. 34, No. 873, Sept., 1911, p. 384.

In speaking of the flies from this time on, I shall use the letter *N* to signify the presence of the factor for normal body color, *Y* for yellow body color, *R* for red eye, and *W* for white eye. Of these factors, *N* is dominant over *Y*, and *R* over *W*. The factors *N* and *R* are only found in association with the *X*-chromosomes, which is duplex in the female but simplex in the male. This means, of course, that *N* and *R*, as used here, are sex-linked characters. It is not necessary to assume that any of these factors are units but that they act as units in the following experiments.

According to the theory to be tested we have the following data as to the gametic constitution of the flies. A pure *NR* female has the constitution *NRX—NRX*, and all her gametes bear the factors, *NRX*. A pure *NR* male has the constitution *NRX—YW*, and the sperms bear either of two combinations of factors, *NRX* or *YW*. A pure *YW* female has the constitution *YWX—YWX*; the gametes all bear *YWX*. The pure *YW* male has the constitution *YWX—YW*, and his gametes bear either *YWX* or *YW*.

Experiment I.

In experiment I, a pure *NR* female was crossed to a pure *YW* male. The results to be expected in the F_1 and F_2 generations if eye color and body color are independently mendelizing, are shown in Table I.

Table I.

P_1.	*NRX—NRX* = *NR*♀	*YWX—YW* = *YW*♂
	Gametes *NRX*	*YWX*, *YW*
F_1.	*NRX—YWX* = *NR*♀	*NRX—YW* = *NR*♂
	Gametes (1) *NRX*, (2) *YWX*, (3) *YRX*, (4) *NWX*	*NRX*, *YW*
F_2.	*NRX—NRX* = *NR*♀	*NRX—YW* = *NR*♂
	YWX—NRX = *NR*♀	*YWX—YW* = *YW*♂
	YRX—NRX = *NR*♀	*YRX—YW* = *YR*♂
	NWX—NRX = *NR*♀	*NWX—YW* = *NW*♂

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[1] *Science*, July 22, 1910, p. 120; *Science*, March 31, 1911, p. 496; *Jour. Exper. Zoöl.*, November, 1911, p. 365.

This table shows that all members of the F_1 generation should have the appearance of normal *NR*'s. In my experiments, this was true without exception.

The females of the F_1 generation should produce four classes of eggs, representing all combinations of *N*, *R*, *Y* and *W*. The males should produce sperms of two classes. The combination of these gametes should produce in the F_2 generation equal numbers of males and females, but all of the females should be *NR*, while one fourth of the males should belong to each of the following classes: *NR*, *YW*, *YR*, *NW*.

If, on the contrary, the *N* and *R* are completely coupled we have the same result in the F_1 generation, but the gametes of the F_1 females would be of two classes only, viz., *NRX* and *YWX*, while those represented in the table by (3) and (4) would be absent. Therefore there would occur in the F_2 generation *NR* females as before, and only two kinds of males, one half being *NR* and the other half *YW*.

Table II. shows the actual results of this experiment. From 24 bottles I raised 11,394 flies. Of these 6,081 were females. All but one of these females were *NR*. The other was *NR* on the left side and *YW* on the right, and may be for present purposes ignored[1] as an anomaly. Of the males there were of class *NR*, 2,870; class *YR*, 34; class *NW*, 36; class *YW*, 2,373. Whatever the explanation may be, it is obvious that coupling has occurred. It is equally obvious that the coupling is not complete.

The result for the individual bottles accorded with that of the total. There is in every bottle except number 6, a larger number of *NR* males than of *YW* males. This is without doubt due to a greater vitality in the normal flies than in the mutants, for also in the pure cultures the normal flies are much more fertile than the others. This matter is discussed by Morgan in one of the papers mentioned[2] above, under the heading, "The Fertility of Deficient Mutations." I have no reason to dissent from the statements made there. The reasons for this infertility are now being investigated. At present, at least, the fact is very apparent, not only in my own results, but also in those published by Morgan.

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[1] This fly was active and except for color appeared to be normal. I attempted to mate her to *YW* males, but she appeared to be sterile, and after two weeks she was preserved for histological study.

[2] *Jour. Exper. Zoöl.*, Nov., 1911.

TABLE II.

TABLE SHOWING RESULTS OF EXPERIMENT I.

Bottle No.	*NR* ♀.	*NR* ♂.	*YR* ♂.	*NW* ♂.	*YW* ♂.	Total.	
1	201	92	4	3	81	381	
2	161	89	1	1	77	329	
3	282	123	1	1	90	497	
4	351	155	0	3	127	636	
5	46	28	1	1	18	94	
6	96	37	0	0	40	173	
9	108	46	1	0	33	188	
10	267	147	5	0	126	545	
13	253	144	0	1	84	482	
14	183	104	1	1	72	361	
15	253	144	1	1	135	534	
17	317	143	1	2	123	586	
19	352	165	1	2	132	652	
20	292	121	3	3	104	523	
21	277	146	2	1	102	528	
22	280	114	0	0	99	493	
23	136	63	0	0	72	271	
24	160	80	0	1	59	300	
25	127	73	2	0	55	257	
26	242	118	0	0	64	424	
27	544	234	3	2	233	1,016	1 abnormal ♀
50	503	203	5	8	153	872	$\frac{1}{2}$*NR*, $\frac{1}{2}$*YW*
51	325	154	0	3	148	630	
52	324	147	2	2	146	621	
Totals	6,080	2,870	34	36	2,373	11,393	11,394

EXPERIMENT II.

In this experiment, the reverse cross was made, viz., pure *YW* females by pure *NR* males. According to the theory if no coupling occurred, the F$_1$ and F$_2$ generations should give the results shown by Table III.

TABLE III.

P$_1$.	*YWX—YWX* = *YW* ♀	*NRX—YW* = *NR* ♂
	Gametes *YWX*	*NRX*, *YW*
F$_1$.	*NRX—YWX* = *NR* ♀	*YWX—YW* = *YW* ♂
	Gametes (1) *NRX*, (2) *YWX*, (3) *YRX*, (4) *NWX*	*YWX*, *YW*
F$_2$.	*NRX—YWX* = *NR* ♀	*NRX—YW* = *NR* ♂
	YWX—YWX = *YW* ♀	*YWX—YW* = *YW* ♂
	YRX—YWX = *YR* ♀	*YRX—YW* = *YR* ♂
	NWX—YWX = *NW* ♀	*NWX—YW* = *NW* ♂

The table shows that in the F_1 generation there should be only *NR* females and *YW* males. My studies completely bore out this conclusion.

As in the previous experiment, the females of the F_1 generation should produce four classes of eggs, representing the various combinations of the factors concerned. The males should produce two classes of sperms. The union of these gametes should produce in the F_2 generation equal numbers of males and females of the four classes, *NR*, *YW*, *YR*, *NW*.

Complete coupling would give only two classes of eggs and hence only two classes of males and females, viz., *NR* and *YW*. Table IV. shows the results actually obtained. From 24 bottles there hatched 9,626 flies, distributed as follows: *NR* females, 2,761; *NR* males, 2,462; *YR* females, 30; *YR* males, 29; *NW* females, 21; *NW* males, 24; *YW* females, 2,204; *YW* males, 2,095.

Here again the coupling occurs, and again the coupling is not complete. We find also the deficiency of males and of mutants

Table IV.

Giving Results of Experiment II.

Bottle No.	*NR* ♀.	*NR* ♂.	*YR* ♀.	*YR* ♂.	*NW* ♀.	*NW* ♂.	*YW* ♀.	*YW* ♂.	Total.
7	101	74	0	0	0	4	102	76	357
8	46	48	2	0	0	0	40	36	172
11	63	63	0	0	3	0	64	68	261
12	87	80	1	2	1	0	67	75	313
16	74	88	1	0	0	3	79	80	325
29	82	81	0	1	1	1	86	77	329
31	206	168	1	5	0	1	176	161	718
32	126	111	0	2	1	3	122	120	485
33	139	130	5	1	0	2	112	137	526
34	153	152	1	1	2	1	116	101	537
35	76	42	0	0	0	3	19	31	171
36	78	55	0	1	0	1	56	51	242
37	75	74	1	0	0	0	56	64	270
39	48	39	0	2	0	0	30	24	143
40	46	45	0	0	0	0	41	43	175
54	114	103	4	4	2	0	96	81	404
55	119	100	1	1	1	1	78	74	375
56	178	156	2	1	3	1	138	153	632
57	137	118	1	2	2	0	118	104	482
58	183	181	3	3	1	0	152	130	653
59	184	143	0	0	1	0	139	100	567
60	202	190	5	2	1	0	160	156	716
61	195	153	1	1	1	3	121	124	599
62	49	58	1	0	1	0	36	29	174
Total	2,761	2,462	30	29	21	24	2,204	2,095	9,626

when compared with the normal females. The figures in either of these experiments show an average of about eighty-five individuals in which the coupling persists to one in which the factors are interchanged.

Experiment III.

After carrying the above experiments to near their conclusion I suspected that perhaps there was some reason unknown to me which made N naturally couple with R, no matter what the original combination might have been. That my suspicions were ill-founded was demonstrated by the following experiments.

In the first of these, I selected from the classes of NW females and YR males of the F_2 generation (Table III.) obtained in Experiment II., virgin individuals and mated them. The gametic constitution of these and the nature of their offspring according to the theory are shown in Table V.

Table V.

The P_1 Generation of this Table Consists of Members of the F_2 Generation of Experiment II.

P_1. NWX—YWX = NW♀ YRX—YW = YR♂
Gametes NWX, YWX YRX, YW
F_1 (1) NWX—YRX = NR♀ (3) NWX—YW = NW♂
(2) YWX—YRX = YR♀ (4) YWX—YW = YW♂

This table shows that equal numbers of NR females, YR females, NW males, and YW males should be produced. I obtained:

NR females, 45 NW males, 52
YR females, 50 YW males, 47

I now took some of the virgin females from the NR group and inbred them to the YW males. It should be recalled that the parents of the F_2 generation in Experiment II. were also of these classes, and therefore, as shown by Table VI., if body color and eye color were not to couple, we should expect the same classes of individuals in the F_2 generation in each case. If coupling occurred here (*i. e.*, if having "broken" once, should not give any tendency to do so again), we should expect here, not the same results as those obtained in the previous experiment, but rather that the classes that were then small should now be the large classes, while the large ones should be now small.

I started six bottles of this cross, and in every case the classes *YR* and *NW* were in the majority. But in five of the bottles the food was very wet and the hatch poor. None of them gave any *YW*'s at all. Also, in every case the class *NW* far outnumbered the class *YR*. In the sixth bottle (see Table VII., Bottle No. 44) the food was in better condition (*i. e.*, was drier), the hatch larger, the ratios nearer the expected, and class *YW* represented. This would seem to indicate some relation between the dark body color and the relative dampness of the food, though I realize that this suggestion requires further investigation.

TABLE VI.

The members of F_1 are taken from classes (1) and (4) of Table V.

F_1. *NWX—YRX* = *NR*♀   *YWX—YW* = *YW*♂
Gametes (1) *NRX*, (2) *YWX*,   *YWX*, *YW*
(3) *YRX*, (4) *NWX*

F_2. *NRX—YWX* = *NR*♀   *NRX—YW* = *NR*♂
YWX—YWX = *YW*♀   *YWX—YW* = *YW*♂
YRX—YWX = *YR*♀   *YRX—YW* = *YR*♂
NWX—YWX = *NW*♀   *NWX—YW* = *NW*♂

TABLE VII.

GIVING RESULTS OF EXPERIMENT III.

Bottle No.	*NR*♀.	*NR*♂.	*YR*♀.	*YR*♂.	*NW*♀.	*NW*♂.	*YW*♀.	*YW*♂.	Total.
41	1	1	17	19	63	37	0	0	138
42	2	1	10	6	66	55	0	0	140
43	1	0	3	1	44	47	0	0	96
44	0	2	91	90	103	115	2	0	403
45	1	1	9	13	41	25	0	0	90
46	0	3	11	7	74	40	0	0	135
Total	5	8	141	136	391	319	2	0	1,002

That the coupling here is in accord with that of the previous experiments is clear. A study of bottle number 44 in which the food was not wet and sticky will present an even fairer view of the case, as well as one in which the results are nearer those expected.

EXPERIMENT IV.

Here again I selected from virgin members of the F_2 generation of Experiment II., this time crossing *YR* females and *NW* males.

Their supposed gametic constitution and the results to be expected in the F_1 generation are shown in Table VIII.

Table VIII.

The P_1 generation consists of members of the F_2 generation of Experiment II. (see Table III.).

P_1. *YRX—YWX* = *YR*♀ *NWX—YW* = *NW*♂
Gametes *YRX*, *YWX* *NWX*, *YW*

F_1. (1) *YRX—NWX* = *NR*♀ (3) *YRX—YW* = *YR*♂
(2) *YWX—NWX* = *NW*♀ (4) *YWX—YW* = *YW*♂

The table shows that there should be produced in equal numbers *NR* females, *NW* females, *YR* males, and *YW* males. The actual hatch consisted of

NR females,	116	*YR* males,	82
NW females,	116	*YW* males,	100
		NW males,	2

I am quite unable to account for the occurrence of the two *NW* males, except through mutation. Rather than offer this rather wild guess, I shall not attempt to explain it at all. It will be observed, however, that it could not possibly have been that the *YR* females were not virgin when the experiment was begun, for no known mating will produce an *NW* male from such a female. Also, it would be an unbelievable coincidence that should produce on the same day—the fifth after the beginning of the hatch—two such males on account of contamination of the food.

As in experiment III., I mated the virgin *NR* females to the *YW* males, so that Table VI. accounts for the results in the F_2 generation in the same way that it did for experiment III. The discussion, also, of the probabilities for the F_2 generation in that experiment applies equally well to this experiment.

I started only one bottle of this kind (No. 47). From it were produced:

NR females, 2;	*YR* females, 81;	*NW* females, 89;	*YW* females, 1;
NR males, 1;	*YR* males, 82;	*NW* males, 90;	*YW* males, 0.

A comparison of these figures with those of bottle No. 44 of experiment III. will show a very close agreement of the one to the other.

Discussion of the Problem in the Light of the Experiments.

In the above experiments we find Morgan's observations verified.

1. A coupling occurs in the F_2 generation in which the coupled factors are those that were associated in the grandparents.
2. This coupling is by no means complete.
3. The females of any class as a rule outnumber the males.
4. The classes which lack the factor for red eye or normal body color contain a smaller number of individuals than those which possess this factor, and the class lacking both of them is the smallest of all.

Perhaps the last two conclusions may be stated: The vitality of any class of individuals is increased according to the number they possess of factors found in the normal (*NR*) female. On this assumption, the reason why the *NR* males are less numerous than the *NR* females would be accounted for by their having the simplex rather than the duplex gametic constitution. Perhaps an individual has an added vitality even through the possession of two rather than one of the factors for sex, as in the case of the *YW* females as opposed to the *YW* males. I do not desire to urge the case, particularly since I understand Morgan is conducting investigations that will throw more light on the subject.

The particular problem presented by these experiments is to explain why the coupling occurs but is not complete. This problem perhaps can be finally settled only by the cytologist.

Morgan's suggestion, based on the observations of Jannsens, on the twisting of the chromosomes, I understand as follows. The factors through the influence of which any particular character appears in an individual, are represented in the chromosomes by material particles which have always the same relative linear position. "When the parental pairs (in the heterozygote) conjugate, like regions will stand opposed. There is good evidence to support the view that during the strepsinema stage, homologous chromosomes twist around each other, but when the chromosomes separate (split), the split is in a single plane."[1]

---

[1] Morgan, in *Science*, Sept. 22, 1911, p. 384.

Now the supposition is that if two of the factors lie close together on the same side of the double chromosome, they may escape separation by the splitting. This means that the factors must be closer together than half the distance around one complete turn of the spirally twisted chromosomes. This length is the maximum possible in order to allow two factors to be coupled on account of proximity. This length might be indefinitely diminished so that many factors of one of the parental chromosomes might lie closely approximated on the same side of the split, and therefore be coupled.

In order to make the situation clearer to myself, I took two pieces of one fourth inch rubber tubing, eighteen inches long, and marked off, with ink, lengths of one inch. Each tube represented one chromosome of a homologous pair, and the inch lengths represented the factors. I marked the factors on one chromosome with odd numbers, 1, 3, 5, 7, etc., to 35. This chromosome I called maternal. The factors of the other I marked with 2, 4, 6, 8, etc., to 36, and called this chromosome

TABLE IX.

The two vertical columns named *r* (right) and *l* (left) contain all the factors of both parents. Each column represents one gamete and contains that half of the factors not found in its fellow. Odd numbers represent maternal, even numbers paternal characters.

r	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*
No turn		$\frac{3}{8}$ turn		$\frac{1}{2}$ turn		turn $\frac{5}{8}$		$\frac{3}{4}$ turn		$\frac{7}{8}$ turn		One turn	
1	2	1	2	1	2	1	2	1	2	1	3	1	2
3	4	3	4	3	4	3	4	3	4	3	4	3	4
5	6	5	6	5	6	5	6	5	6	5	6	5	6
7	8	7	8	7	8	7	8	7	8	7	8	7	8
9	10	9	10	9	10	9	10	9	10	9	10	9	10
11	12	11	12	11	12	11	12	11	12	11	12	12	11
13	14	13	14	13	14	13	14	14	13	14	13	14	13
15	16	15	16	15	16	16	15	16	15	16	15	16	15
17	18	17	18	17	18	18	17	18	17	18	17	18	17
19	20	19	20	20	19	20	19	20	19	20	19	20	19
21	22	21	22	22	21	22	21	22	21	22	21	22	21
23	24	24	23	24	23	24	23	24	23	24	23	24	23
25	26	26	25	26	25	26	25	26	25	26	25	25	26
27	28	28	27	28	27	28	27	28	27	28	27	27	28
29	30	30	29	30	29	30	29	30	29	29	30	29	30
31	32	32	31	32	31	32	31	32	31	31	32	31	32
33	34	34	33	34	33	34	33	34	33	33	34	33	34
35	36	36	35	36	35	36	35	36	35	35	36	35	36

paternal. Thus 1 and 2 represented the two members of a pair of homologous factors, as did also 3 and 4, 5 and 6, etc., to 35 and 36, in every case the odd number representing the maternal, and the following even number the corresponding paternal factor.

I now secured the ends of the chromosomes so that the proper factors stood opposed to each other and began to twist the chromosomes.

It was at once apparent that if the twisting should not vary in the members of one species, coupling would be invariable, nor would the coupling be that representing either parent alone, but both, and the combination of factors would be always the same. This is shown in Table IX. In this table the vertical columns represent the gametes formed when a splitting follows the number of twists named at the top of the column. In each case there are two different combinations of gametes; one formed on the left and one on the right side of the split.

A study of the above table will show that if there be, for instance, no turn and the split falls between the two chromosomes all the factors in the gamete on the right will be maternal. If there be three eighths of a turn, the odd-numbered factors from 1 to 21 (maternal) and the even-numbered factors from 24 to 36 (paternal) will always be in the gamete on the right. And so in every case, if the amount of twist be constant, the factors present in any gamete will be constant. This will be true even if the factors are of different lengths, and the twisting not uniform for all parts of the same chromosome, if only the lack of uniformity be constant in all the members of the species.

Now, the nature of the twisting and the amount of variation that occurs can only be solved, if at all, by the cytologist. On the face of the question as so far presented it must be that variation in the twist occurs, or there would, on the basis of the theory here discussed, be no such thing as "independent mendelizing," but a constant coupling. My question is therefore, "What are the facts concerning this twisting? How uniform is it?" For I conceive it to be possible that if the twisting be nearly definite, coupling of certain factors would generally occur, and would depend not entirely on the nearness together of the factors, but on the amount of the twist, and on the side of the splitting double chromosome on which any factor should be thrown by the twist. As Morgan points out, the chances of separation are not so great for factors which lie near each other as for those far from each other, though coupling would not necessarily imply that the factors should lie in one segment of the twisted chromosome. According to this idea the factors for wing length, as explained by Morgan in the paper in the *Journal of Experimental Zoölogy* for November, 1911, may not be closely approximated to those for eye color and body color.

Summary.

1. The factors for red and for white eye color and for normal and yellow body color in *Drosophila* have been the subjects of investigation in these experiments.

2. In the F_2 generation the factors for eye color and body color appear to a great extent associated in the same combinations that were present in the grandparents, the interchanging taking place only once to nearly eighty cases where there is no interchanging.

3. The absence in a fly of certain characters found in the normal wild fly, seem to render it less fertile, or at least such flies hatch in smaller numbers than do those in which such characters are present.

4. These facts are in accord with the principles and theories advanced by Morgan, and can be explained on a theory based on (1) the relative positions of factors in the chromosomes, (2) the twisting of homologous chromosomes about each other in gametogenesis, and (3) their subsequent splitting in one plane.

5. Further cytological evidence bearing on the above theory is much to be desired.

SIZE RELATIONSHIPS BETWEEN CONJUGANTS AND NON-CONJUGANTS IN BLEPHARISMA UNDULANS.

FLORENCE A. WATTERS.

It has been shown by Pearl ('07) that in *Paramœcium* cultures the conjugants differ from the non-conjugants in size and variability and that a correlation in size occurs between the two members of conjugating pairs. He has shown by statistical methods (1) that the conjugants are less variable in size than the non-conjugants, (2) that the conjugants show a smaller mean size than the non-conjugants, and (3) that a marked correlation in size exists between the members of the conjugating pairs, the smaller uniting with the smaller and the larger with the larger.

Jennings ('11) took up the problem of size relationships in *Paramœcium* going into discussion of the reasons for the conditions found, which he based on very careful observations of the conditions existing in his cultures. After careful measurement of a great many individuals he confirmed the results obtained by Pearl, *i. e.*, (1) that the conjugants are smaller than the non-conjugating population of a culture; (2) that they are less variable than the non-conjugants; and (3) that there is a marked correlation in size between the members of the pairs, so that on the whole larger individuals are found mated with larger, smaller individuals with smaller. It was with the idea of investigating the size relationships in another related form, that the present work on *Blepharisma undulans* was taken up. It will be possible here to give the facts of the size relationships only as they were found in material already mounted.

The individuals under consideration are from a pure-line culture isolated at Woods Hole on July 20, 1911, by Professor Calkins. The six lots with which I shall deal were killed by him at various times during October, 1911, by means of sublimate acetic, stained with Hoyer's picrocarmine, and mounted permanently in Canada balsam.

In measuring the individuals, a Leitz projectoscope was used to throw a projection of each one upon the drawing table below. A permanent record of each one was obtained by drawing a line perpendicular to the long axis at each end. The figures given are the measurements of the projections rather than of the individuals themselves and are 192 times the actual size. Thus in the table:

	Longest, Mm.	Shortest, Mm.	Average, Mm.
Non-conjugants	42.5	13.5	27.67
Conjugants	30.0	15.5	20.12

the size of the projection rather than the actual size is given. Translated, these figures would be:

	Longest, μ.	Shortest, μ.	Average, μ.
Non-conjugants	221.35	70.31	144.11
Conjugants	156.25	78.12	104.79

But since it is the relative length rather than the absolute length which is of importance in this case, it has not seemed necessary to divide all the numbers given below by 192, and the figures denoting the size of the projection stand in the statistics which follow. For the sake of overcoming any element of personal error which may have entered into the measurements, all measurements falling half way between one mm. mark and the next are considered with those .5 mm. larger.

The results obtained by careful measurements of six lots of *Blepharisma undulans* are in accord with the results obtained by Pearl ('07) and Jennings ('11), *i. e.*, in *Blepharisma undulans* (1) the mean length of the conjugants is less than the mean length of the non-conjugants, (2) the variation in length is much less among the conjugants than among the non-conjugants, and (3) the correlation in size between the two members of a pair is distinctly marked, the larger mating with the larger and the smaller with the smaller. To take up first the difference in length between the conjugants and non-conjugants: Table I. shows that in all six lots the mean length of the conjugants is less than that of the non-conjugants.[1] Plate I. shows that the mean length of the

[1] The ordinates indicate the size of the individuals while the abscissas indicate the number of individuals; thus the position of the column indicates the size while the height of the column indicates the number of individuals.

conjugants of lot 1 is 21 units and that the number of individuals increases rather rapidly from the extremes, 17 and 30, toward the mean, 21; also that the mean length of the non-conjugants is 29 and that the number of individuals increases slowly at first and then more rapidly from the extremes 18 and 40 to the mean 29. The average lengths of the conjugants and non-conjugants is in accord with the mean length, being greater in the case of the non-conjugants and less in the case of the conjugants (Table I.).

Plate II. shows that the mean length of the conjugants of lot 2 is less than the mean length of the non-conjugants, the former being 22 and the latter 32. The number of individuals of the conjugants and non-conjugants increases from the extremes 18.5 and 28 in the case of the former, and 18.5 and 42.5 in the case of the latter, to the means 22 and 32. That the increase from extremes to means is much more gradual in lot 2 than in lot 1, is due to the fact that there are over twice as many individuals in lot 1 as in lot 2. The average length of the conjugants is less than that of the non-conjugants, as in lot 1, being 23.46 and 33.88 respectively.

TABLE I.

SIZE RELATIONS IN CONJUGANTS AND NON-CONJUGANTS.

Series.		I.	II.	III.	IV.	V.	VI.	Total.
No.	N.	390	148	551	47	47	198	1,381
	C.	160	30	292	20	20	28	550
Total length	N.	10,898	5,014.5	13,744	1,253	1,112.5	6,195	38,217
	C.	3,426.5	704	6,057.5	415.5	431	638.5	11,673
Average	N.	27.9	33.88	24.94	26.65	23.67	31.28	27.67
	C.	21.41	23.46	20.06	20.77	21.55	22.80	20.12
Longest	N.	40	42.5	33.5	34.5	34	40	42.5
	C.	30	28	27	25	25.5	27.5	30
Shortest	N.	18	18.5	13.5	18	18.5	16.5	13.5
	C.	16.5	18.5	16.5	15.5	18.5	19	15.5
Variations	N.	22	24	20	16.5	16.5	23.5	29
	C.	13.5	9.5	10.5	9.5	7	8.5	14.5
Mean	N.	29	32	25	29	27	32	27.5
	C.	21	22	21	20	22	23	21
Median	N.	29	30.5	23.25	26.25	26.75	28.25	28
	C.	23.25	23.25	21.25	20.25	22	23.25	22.75

N. indicates non-conjugants.

C. indicates conjugants.

Lot 3, Plate III., shows the same relative conditions as to mean and average length of conjugants and non-conjugants, the mean length of the former being 21, and that of the latter 25. The averages are 20.06 and 24.94 respectively. The rapid increase in the number of individuals from the extremes 16.5 and 27, and 13.5 and 33.5 respectively is due to the larger number of individuals present in lot 3.

Lot 4, Plate IV., shows the same relative conditions as those found in the other lots, the mean lengths of conjugants and non-conjugants being 20 and 29 respectively, while the average lengths are 20.77 and 26.65. The small number of individuals occurring in lot 4 accounts for the lowness of the columns.

Lot 5, Plate IV., is like lot 4 in the number of individuals and in the shortness of the columns. There is greater irregularity of outline in lot 5 than in any other of the six lots, but the mean length of conjugants and non-conjugants is in accord with the mean length of the other lots, the former being 22 and the latter 27.

Lot 6, Plate IV., agrees with the rest in having the mean and average lengths of the conjugants smaller than those of the non-conjugants, the means being 23 and 32, and the averages 22.80 and 31.28 respectively. Thus in every one of the six lots, the mean and average have been less in the case of the conjugants than in that of the non-conjugants, the difference being from 5 to 10 units in the case of the means, and from 2 to 10 in the case of the averages. From all this we should expect to find that the mean and average of the total number of conjugants are less than those of the total number of non-conjugants. This is the case, the mean for the total number of conjugants being 21 and the average 20.12; and the mean for the total number of non-conjugants being 27 and 28 (or 27.5) and the average 27.67.

One interesting fact appears in Table I. and Plates I. to IV., which is shown best in Plates IV. and V., *i. e.*, the population including conjugants and non-conjugants shifts toward the left or right in the various lots. Plate V. shows this for the non-conjugants of lots 1, 2, and 3, the means being 29, 32, and 25, and the extremes 18 and 40, 19 and 43, and 14 and 34 respectively. The means of the non-conjugants of lots 4, 5, and 6, Plate IV., are 29, 27, and 32 respectively while the extremes are 18 and 35, 19 and 34, and 17 and 40. The conjugating populations do not show this so well since they vary less than the non-conjugating populations.

This brings us to the consideration of the second of the three problems, *i. e.*, the variation of conjugants and non-conjugants. Table I. shows very clearly that the variation between the extremes in the case of the conjugants is much less than that in the case of the non-conjugants, being in the six lots:

13.5	9.5	10.5	9.5	7	8.5	in the former,
22	24	20	16.5	16.5	23.5	in the latter,

the variation ranging from nearly two times to nearly three times as great in the case of the non-conjugants. It is probable that some of the shortest non-conjugants are ex-conjugants, which would however not change the general results very appreciably.

Finally the third problem, as to coördination in size of the members of the same pair, appears. By plotting a graph using the length of the shorter of the two individuals as abscissa, and the longer as ordinate, the number of pairs of each combination which appeared was obtained. Then by adding diagonally, I obtained the number of pairs in which the individuals varied less than .5 mm. (or 2.2 microns, actual measurement), which varied .5 mm., 1 mm., 1.5 mm., etc., the following table resulting:

TABLE II.

CORRELATION IN SIZE OF MEMBERS OF CONJUGATING PAIRS.

	Pairs.
Both members equal in length	35
One member shorter by 0.5 mm	57
One member shorter by 1.0 mm	62
One member shorter by 1.5 mm	50
One member shorter by 2.0 mm	34
One member shorter by 2.5 mm	17
One member shorter by 3.0 mm	9
One member shorter by 3.5 mm	8
One member shorter by 4.0 mm	2
One member shorter by 4.5 mm	2
One member shorter by 5.0 mm	2
One member shorter by 5.5 mm	1

No two members of the same pair show a greater variation than 5.5 mm., though conjugating individuals of different pairs show as great a variation as 14.5 mm.; and out of 279 pairs of conjugants only 7 pairs show a greater variation than 3.5 mm. This indicates a very definite correlation of size between the two members of the conjugating pairs.

The same conditions which Pearl ('07) and Jennings ('11) found to exist in *Paramœcium*, then, have been shown to exist in the case of *Blepharisma undulans*, *i. e.*, (1) the mean length of the conjugants is less than that of the non-conjugants, (2) the variation of the conjugants is less than that of the non-conjugants, and (3) there is a definite positive correlation in size between the members of the conjugating pairs, the larger uniting with the larger individuals and the smaller with the smaller.

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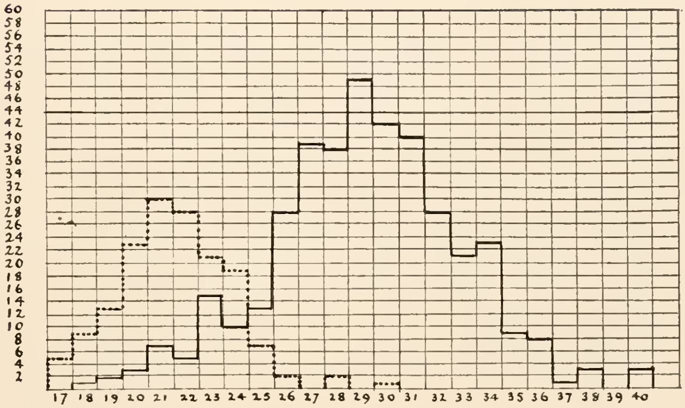
EXPLANATION OF PLATES.

PLATE I.

Lot 1. *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugates. ..... conjugants.



FLORENCE A. WATTERS.

Plate II.

Lot 2. *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.

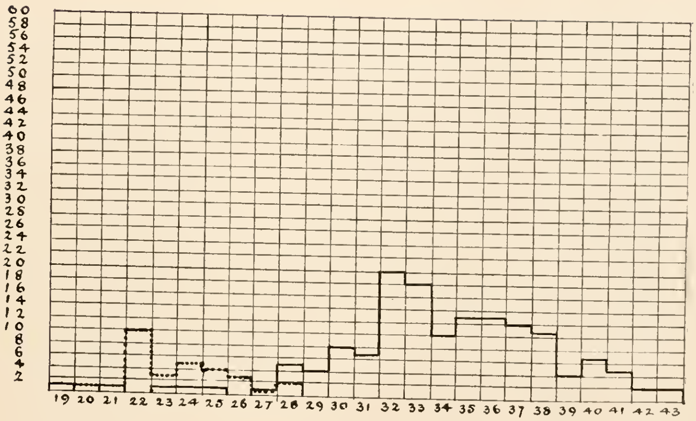


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PLATE III.

Lot 3. *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.

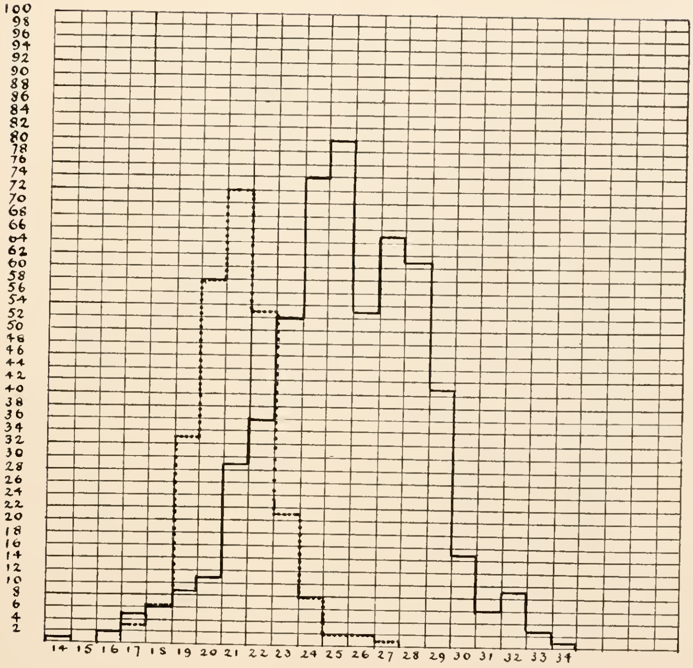

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PLATE IV.

Lots 4, 5, 6. *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.

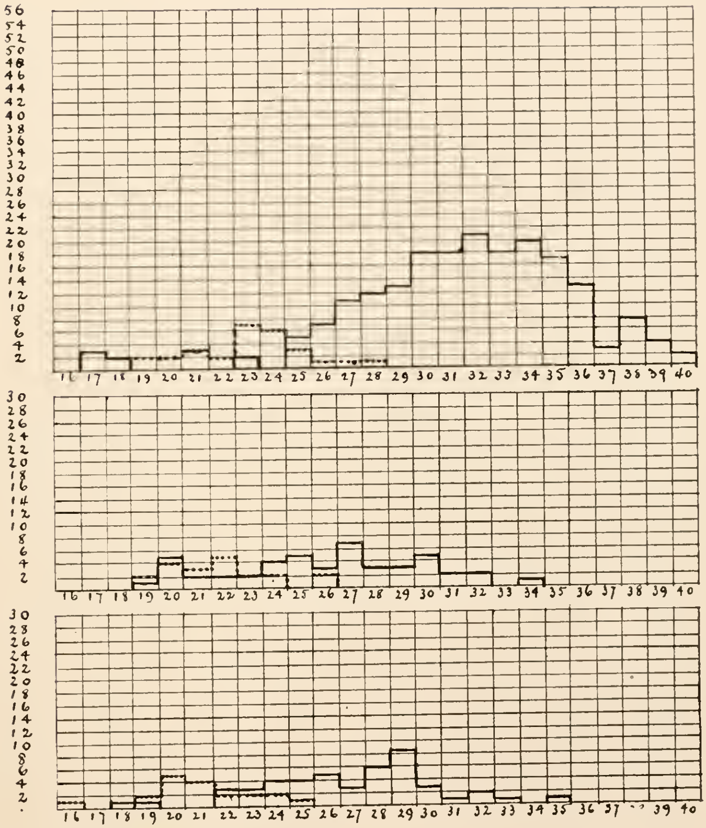

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PLATE V.

Lot "Total." *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — non-conjugants. ..... conjugants.

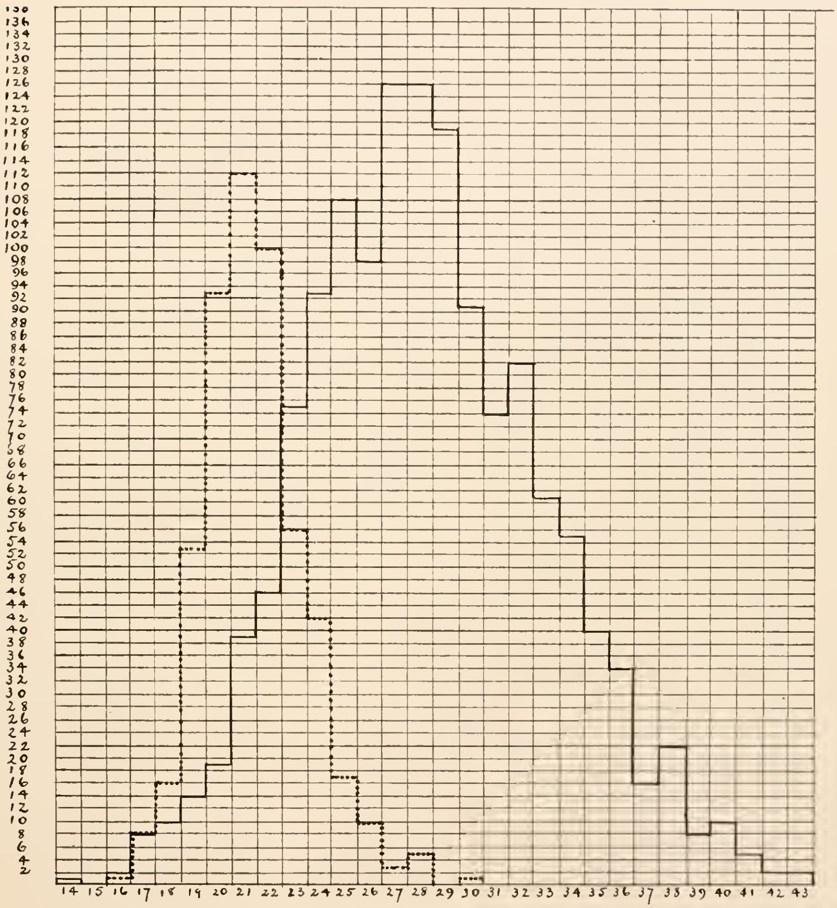

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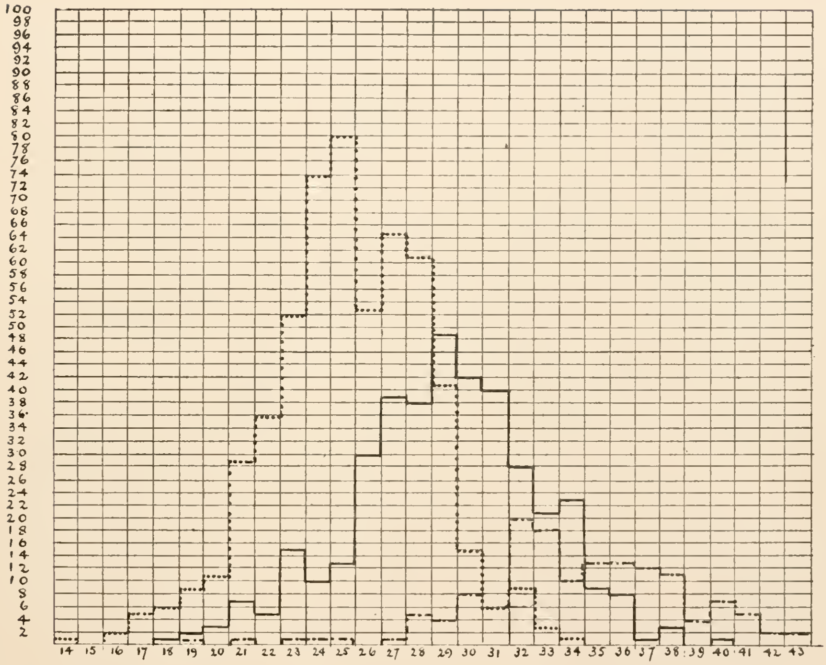
PLATE VI.

Lots 1, 2, 3. *Blepharisma undulans.* Abscissas: number of individuals. Ordinates: size of individuals. — Lot 1. ..... Lot 2. — · — Lot 3.



FLORENCE A. WATTERS.

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BIOLOGICAL BULLETIN

A CONTRIBUTION TO OUR KNOWLEDGE OF THE FUNCTION OF THE ŒNOCYTES OF INSECTS.[1]

R. W. GLASER.

The function of the cells known as the œnocytes has been the object of a considerable amount of speculation by various investigators. The studies which constitute the basis of these speculations have all been of a morphological character and, while valuable in their way, throw no light on the physiology of these very singular elements. So much has been written about them, especially about their morphology, that we will consider only a few of the more interesting views on their possible function. In 1873 Graber called attention to the fact that the œnocytes are glands secreting a substance concerning which nothing is known. Later ('91) he supposed that they are metamorphosed into the fat-body, and also give rise to blood corpuscles. This was corrected by Wheeler, in 1891, who through a study of the embryological development of these cells, concluded that they neither give rise to the fat-body nor to the blood. Pantel ('98) and Berlese ('99) endowed the œnocytes with an excretory function, the latter supposing that they serve during the periods of moulting and pupation when the Malpighian tubules are functionless. Anglas ('00) advanced the view that the œnocytes may possibly secrete ferments. Koschevnikov ('00) makes the remarkable statement, among others, that he has preparations which show plainly that the œnocytes swallow fat cells. He further says, "unnecessary substances which get into the blood stop in the interior of the œnocytes in the form of granules. The cells are not periodically emptied of this excretion product, but are continually being filled. Finally they are completely filled and are made entirely useless for further activity. It may be that the inability of the œnocytes for further work brings about a disturbance of the regular metabolism, and is hence one of the causes of the sinking life activity of the insect." It seems as if Koschevnikov must have confused the œnocytes with phagocytes; at any rate he did not study the œnocytes. Janet ('07) regards the œnocytes as real unicellular glands. "Like these they take from the blood elements for their functional activity. The substance resulting from this activity they give up by osmosis to the adipocytes which use it possibly for the elaboration or dissolution of reserves, possibly for the production of a special reserve substance." Verson ('11) found at times at the periphery of the cells microscopical exudations of a particular excretion which was accompanied by changes in the form and size of the nucleus. Gee ('11) injected specimens of *Platyphylax designatus* larvæ with methylene blue, and found that immediately after injection the œnocytes and spinning glands both took the stain, the œnocytes less than the spinning glands. "The Malpighian tubules, except in one case, did not take the stain. In larvæ killed half an hour after injection the œnocytes were more deeply stained, but no coloration was observed in the Malpighian tubules. Larvæ killed one hour after injection showed that the Malpighian tubes had begun to take up the blue color, but that the œnocytes and spinning glands were becoming less intense, in coloration." He reached the conclusion that the similar reaction of œnocytes and spinning glands towards methylene blue seems to show that the œnocytes are secretory rather than excretory, the spinning glands being secretory. "The nature of their secretion is difficult and practically impossible to determine. Can it be that their function is the secretion of a substance or enzyme which is of aid to the fat-body in its constructive work?"

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 57.

It will be seen from this short review that there are many and diverse opinions as to the function of the œnocytes. On reviewing the literature on the morphology of these cells, the divergence of views is seen to be still greater, no two people agreeing in more than a few points. The insects used by the various investigators were different species and very often insects belonging to distantly related orders. Of course, the form and body orientation of the œnocytes is quite different in non-related forms and even in members of the same species at different periods of their life history. This is exactly what we should expect to find. In numerous cases, however, the varied opinions can be attributed to the fact that many of the investigators did not study the same cells.

I have made sections of larvæ of Trichoptera, Lepidoptera and Diptera and find that the differences between their œnocytes are merely differences of size, shape, density of granulation and amount of ramification of the nuclei. Generally speaking the œnocytes are large, yellow, more or less isolated cells, so large in fact that in some forms they can be readily identified with a pocket lens. They are located in the abdominal segments and in such only as bear spiracles. Here the œnocytes are situated behind the tracheæ. They do not seem to be definitely attached to the tracheæ and sections do not reveal the intrusion of tracheal filaments into these cells. A cytological study with the orange G and iron hæmatoxylin method shows the cytoplasm to be finely granular and the nucleus to be greatly enlarged and ramified, giving the cells the appearance of being highly active.

To throw any light on the physiology of these cells was a difficult task. Comparatively speaking, they are large, yet in nearly all forms too small to deal with experimentally. To be certain of any test one has to have a bulk of material and further after obtaining it, one must be able to dissect out the organs with ease and be certain at all times that they are the same. Fortunately Mr. James W. Chapman, entomologist of the city of Boston, called my attention to the larvæ of the leopard moth (*Zeuzera pyrina*). The life history of this caterpillar, as worked out by Mr. Chapman, extends over a period of three years, during which time it feeds on practically every species of tree or shrub except conifers, and attains at the end of the third year a very large size, accumulating a great amount of fatty tissue and consequently becoming very heavy. On dissecting some of these larvæ, I found the œnocytes to be so enormous that in a three-year-old larva, a cluster could be recognized with the naked eye. In one and two year old larvæ they are much smaller, for they grow in size with age as do the other organs. In this species, the œnocytes are located in the seven abdominal segments which are just those which bear the abdominal spiracles. Behind these they are situated, occurring in clusters around the tracheæ. There are only two clusters to a segment, one on each side. The number of cells in a cluster varies, from ten to forty or fifty, in different segments. The size also varies considerably in different segments (diameter 175–250 μ) and even in the same cluster, yet the smallest one far surpasses the size of any other cell within the body of the insect.

Through the kindness of Mr. Chapman, I was able to obtain a large amount of material and, following the suggestion of some of the previous investigators, principally Anglas and Janet, I began to work on the hypothesis that the œnocytes are unicellular glands, perhaps secreting a ferment.

A number of tests were made for lipase and oxydase. First, however, to become thoroughly acquainted with the reactions of these enzymes, the pancreases of four hogs were used. Lipase and oxydase are known to occur in these organs, and it was thought advisable to work with them for a time before applying tests to the œnocytes. The pancreas was cut from the hog immediately after it was slaughtered, so as to be certain that it was quite fresh. An extract of the organ was at once preserved in toluol to keep out all bacterial infection. This extract was then diluted with physiological .65 per cent. salt solution, one part of extract to ten parts of saline solution. To two cubic centimeters of this diluted extract one fourth of a cubic centimeter of ethyl butyrate was added, plus a small quantity of lacmoid solution. Purified lacmoid crystals were used and the solution was made as nearly neutral as possible. The extract was, of course, kept under the layer of toluol while the other reagents were being added. The specimen was then put in an incubator at body temperature and kept there for 24 hours. As a control test a second and aliquot portion of pancreatic juice was boiled in order to kill any enzyme. This was treated with the same reagents in exactly the same manner, and put in an incubator for 24 hours. At the end of this time both specimens were taken out and it was found that the liquid in the test tube containing the live enzyme had turned red. The fat-splitting enzyme (lipase) had split the ethyl butyrate into alcohol and butyric acid. The control test retained its former blue tint, namely, that of the lacmoid.

The next thing was to see with how small a quantity of extract and reagents the reaction could be obtained. For this purpose very small glass tubes of equal sizes were blown. For the liquids eye droppers of equal sizes were used. A drop was sucked into the dropper and the glass was graded into four equal parts, so that it was possible by having droppers of exactly the same size and gradation to use for each test the same amounts of liquid. The amounts of the substances were then decreased until two drops of pancreatic extract and one fourth of the amount of one drop of ethyl butyrate plus the lacmoid solution were used. The toluol was never omitted. Control tests were again made each time. The characteristic red color was obtained.

When I was thoroughly satisfied that I had mastered the reaction, I made an extract from the œnocytes of large leopard moth larvæ. Two caterpillars, each measuring about 1¾ inches, were used for each experiment. All of the œnocytes were dissected out in physiological salt solution and rubbed in a very small agate mortar. The extract from the œnocytes plus a small quantity of saline solution which was added equalled two drops. This was taken without any further dilution and, accompanied by control tests, treated in exactly the same way as the small amounts of pancreatic extract had been treated. Six experiments were performed. In not one case did I get an acid reaction. This seems to indicate that lipase or a fat-splitting enzyme is not present in the œnocytes.

It occurred to me that perhaps the fat of insects might be different from vertebrate fat, and that after all the œnocytes might secrete a ferment of some sort, the presence of which the above reagents would not reveal and which might have the power of splitting this fat. I could find in the literature on fats, nothing but the broad statement that all animal fats are triglycerides of oleic, stearic and palmitic acids. The important question for me was therefore to determine whether or not insect fats are triglycerides. This was easily accomplished by making the acrolein test. Fatty tissue was dissected out of larvæ and crushed and the fat extracted with ether. This was filtered and evaporated. An amount of the evaporated fat was then heated gently with some potassium acid sulphate till vapors appeared. These were smelled and the nasty odor of acrolein was at once detected. Control tests were made with lard. Hence, I think, I am justified in saying that insect fats are like other animal fats, and further think it safe to say that since insect fats are triglycerides like vertebrate fats, a fat-splitting enzyme like lipase would react to the reagents used for determining that enzyme in vertebrate fats. It must also be considered that a simple ester like ethyl butyrate is chosen for the test on account of the ease with which even the weakest lipase will decompose it. It is therefore quite reasonable to say that lipases are absent when ethyl butyrate is not acted upon.

The tests for oxidizing enzymes were far more difficult, but in the end the results were positive after the technique had been perfected.

The ordinary way of determining whether one is dealing with a peroxydase or a true oxidizing ferment is by the guajacum tincture method. This method, although repeated trials were made, gave no results so far as the œnocytes were concerned. The guajacum was never decomposed and the H_2O_2 seemed not to be acted upon. As will be seen later, the œnocyte extract really decomposed the H_2O_2, but as I had no efficient indicator, it was practically impossible to tell whether there was any reaction, although the amount of the reagent was decreased in proportion to the amount of the extract. When large amounts of extract from whole caterpillars were used, I found, as did Ostwald ('07), that very often the H_2O_2 was decomposed so violently as to cause the liquid to bubble up in the test tube. When using such large amounts of extract the guajacum is also acted upon and the "Hartzsuspension" turns blue, showing peroxydases to be present also.

The method I applied to the œnocytes and which I am about to describe, gave positive results in so far as it showed that the œnocyte extract contains oxidizing ferments to a much greater degree than any of the other organs or tissues. I was unable to classify these oxidizing enzymes any further and am unable to say whether they are oxydases or katalases. A very delicate method had to be devised, for one must bear in mind that the amount of œnocyte extract which can be obtained even from a large number of larvæ is very small. I have already called attention to the fact that the œnocytes are very large in *Zeuzera pyrina* when compared with those of other forms, yet this statement is merely relative and it must be remembered that from the point of view of the physiological chemist they are really minute organs.

The pancreatic extract of the hog was again used and the amounts of the extract and reagents were again decreased in order to train the eye as before to light reactions. Training the eye was hardly necessary in either case. The reaction for lipase was decidedly negative while the reaction for oxidizing ferments was decidedly positive. It was thought safer, however, to take these precautions.

It might be well here to say something concerning the reagent, which was that employed by clinical workers for the demonstration of occult blood. This reagent consists of: 100 c.c. of a 20 per cent. solution of NaOH + 2 grs. phenolphthal*ein* + 10 grs. zinc dust. This is boiled slowly till the solution is decolorized. The fluid is then filtered while still hot into a colored bottle under white petroleum oil. Great care must be exercised to keep out the oxygen of the air, or it will color.

In the experiments one half the quantity of reagent was added to double the quantity of pancreatic extract and a drop or two of a 3 per cent. solution of hydrogen peroxide was added to this. Owing to the fact that oxidizing enyzmes are present in the pancreas, the solution strikes a red color. As control tests water was used instead of extract and treated in the same way. No attention was paid to change of color which develops on prolonged standing. A layer of petroleum was always kept above the specimens to exclude the air.

It was found that the above reagent was satisfactory only so long as a considerable amount of extract was used. When I came down to using very small amounts, *e. g.*, one or two drops, the alkali was entirely too strong and prevented the appearance of a red color. Hence, it proved to be necessary to resort to a finer reagent. The phenolphtha*lin* had first to be isolated as such. A quantity of reagent for occult blood was taken, hydrochloric acid was added till the precipitate that formed had redissolved. The solution was extracted with ether which was washed 3–4 times with small quantities of water to get rid of the acid. The ether was then evaporated without heat in hydrogen. The evaporation in an indifferent gas is safer, although not absolutely necessary, for I find that the phenolphtha*lin* crystals will keep splendidly if stored in very small vials especially when they are filled to the brim before corking so as to exclude the air. Both methods were tried, however. On evaporation one obtains a crust of phenolphtha*lin* which may, if desired, be crystallized from alcohol or a mixture of ether and petroleum ether, which should evaporate spontaneously.

A small piece of phenolphtha*lin* was now placed in a drop of pancreatic extract to which a drop of a ½ per cent. KOH solution and a drop or two of a 3 per cent. solution of hydrogen peroxide had been added. All work was done under oil. The characteristic red color was immediately obtained. Control tests with water were performed each time.

The great quantity of KOH ordinarily used in making the reagent for occult blood is necessary to reduce the phenolphthal*ein* to phenolphtha*lin*. But after this is accomplished the excess of KOH must be gotten rid of as described above, otherwise the color will not develop when such infinitesimal quantities of extract have to be dealt with. A drop or two of ½ per cent. KOH solution seems to be just the proper amount and concentration to obtain the red salt.

In the last experiment with the hog extract two drops of extract were used; a crystal of phenolphtha*lin* was added and to this one drop of ½ per cent. KOH solution plus one drop of hydrogen peroxide. The characteristic red color was immediately obtained. I repeat, as this is very important, that all work was done under oil. Control tests likewise accompanied the actual tests.

Two drops of œnocyte extract were now taken, treated in exactly the same manner as the small amounts of hog extract had been treated, and the immediate reaction was very characteristic. Eighteen experiments of this sort were performed and as all cells have catalytic ferments, other cells as the fat cells, intestinal cells, etc., were submitted to the same tests. When very small amounts of the extract from these cells (*i. e.*, amounts proportional to those of œnocyte extract) were used, it was impossible to determine whether a pink color had developed or not.

Conclusion.

I conclude from the preceding experiments that the œnocytes, which have been regarded by previous investigators as glands, secrete oxidizing enzymes. I do not know whether this is their only function, but it is certainly one of them. At any rate they do not secrete a fat-splitting enzyme. Since these cells, hanging loosely to the tracheæ, lie free in the blood, the enzymes which they secrete may activate the oxygen of the body towards combustion. That the cells actually secrete is indicated by the fact that numerous observers, myself included, have detected microscopical exudations around the periphery of the cytoplasm, especially at times when the nucleus is greatly ramified, and therefore manifesting its great activity.

Exactly what relation the œnocytes bear to the tracheæ, I am unable to say. I saw no definite attachment, but am inclined to believe that a relation exists and that through this the oxydases, one branch of the group of oxidizing enzymes, are able to get their molecular oxygen with the formation of peroxides. It must, moreover, be remembered that Wheeler, in 1892, found the œnocytes of phryganeid larvæ to be provided with delicate processes which are attached to the tracheal hypodermis. That may, of course, be simply a means for attachment and have nothing to do with the passage of oxygen from one to the other. The location of the œnocytes may be purely due to the absence of certain mechanical forces. Wheeler found that "the œnocytes originate by delamination or immigration from the ectoderm, just caudad to the tracheal involutions and after their differentiation from the primitive ectoderm never divide, but gradually increase in size." Since they never divide after differentiation, the mechanical forces of cell division being absent, they are not able to be carried far from their starting point. Moreover, since there are no other growing tissues between them and the ectoderm, except a few small muscles and connective tissue, they are not pressed or shoved out of the way very much, and are so able to retain their original position. I am inclined, however, to the former view, that there is a definite functional relation between these glandular cells and the tracheæ.

That the œnocytes in *Zeuzera pyrina* are so large is probably due to the prolonged larval stage of this insect. As previously stated, it remains a larva three years, during which time it eats ravenously, and grows very heavy, acquiring an enormous amount of fat. Naturally, since it eats so much, and stores up so much reserve food, it has a great deal of material to oxidize, and consequently needs a large supply of oxidizing enzymes. A comparison with other insects would lead me to this view, for those having a short larval stage like the Dipteran larvæ, have much smaller œnocytes in comparison with the remainder of the body than forms with a prolonged larval life, like the phryganeid larvæ, for example.

Before closing, I wish to express my thanks to Professor William M. Wheeler for the kindly advice and encouragement which he has given me at all times. I also wish to thank my father, Dr. Charles Glaser, of Baltimore, for many valuable technical points.

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UPON THE FORMATION OF HYDROCHLORIC ACID IN THE FOVEOLÆ AND ON THE SURFACE OF THE GASTRIC MUCOUS MEMBRANE AND THE NON-ACID CHARACTER OF THE CONTENTS OF GLAND CELLS AND LUMINA.[1]

B. C. H. HARVEY AND R. R. BENSLEY.

CONTENTS.

Introduction 225
Facts which Have Suggested Some Association between the Parietal Cells and the Hydrochloric Acid or its Antecedents 226
  The Prussian Blue Reaction 227
    Fitzgerald's Paper 228
    Our Own Experiments 230
    Summary of the Results Obtained by the Prussian Blue Reaction and their Significance 237
Experiments with Indicators: The Reaction of the Secretion within the Lumen of the Active Fundus Gland and within the Intracellular Canaliculi of the Parietal Cells 239
  Confirmatory Tests 245
The Consistence of the Secretion in the Gland Lumen 247
Other Considerations 248
Bibliography 248

INTRODUCTION.

Ever since it was discovered that hydrochloric acid was produced by the stomach, investigators have been interested in determining the mode and place of its formation. Is this acid produced as such by the gastric glands and even by particular cells of these glands, or do the glands produce only chemical substances which are not themselves acid but which, interacting in the foveolæ or on the surface of the mucous membrane, produce there for the first time the acid as such?

Among those who have sought to discover the origin of this acid, Claude Bernard, Brücke, Lepine, Trinkler, Gmelin and Oppel were unable to find it definitely localized in the glands.

The results of our investigation demonstrate that only non-acid substances are formed by the glands, and that the contents of the gland cells and lumina are not acid, even when hydrochloric acid is being actively formed on the surface of the mucous membrane.

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[1] From the Hull Laboratory of Anatomy, University of Chicago.

In the first part of this paper we shall examine very briefly the evidence which has led some to think that free hydrochloric acid is formed in the parietal cells. We shall consider especially the Prussian blue reaction, which, as used by Miss Fitzgerald ('10), has given by far the most definite results. We shall report some results which we have obtained by this method, which in connection with those reported by her show that this reaction does not prove the presence of free hydrochloric acid in the gastric glands under normal conditions.

In the second part of the paper we shall show that the contents of the parietal cells and of the lumina of the gastric glands are not acid but neutral or alkaline, even when hydrochloric acid is being actively formed on the surface of the mucous membrane.

FACTS WHICH HAVE SUGGESTED SOME ASSOCIATION BETWEEN THE PARIETAL CELLS AND THE HYDROCHLORIC ACID OR ITS ANTECEDENTS.

Heidenhain ('70), Langley ('81) and others were able to associate other secretory functions of the gastric mucous membrane very definitely with other cells. This left the parietal cells and the formation of hydrochloric acid. Further, Miss Greenwood ('85), and afterward Macallum ('08) and Miss Fitzgerald ('10), have shown that chlorides are more abundant in the parietal cells than in other parts of the gland. These facts suggest that there is some association between the formation of the hydrochloric acid and the parietal cells. They leave the question quite open, however, as to where the free hydrochloric acid is first formed as such. The parietal cells probably form substances which later furnish the chlorine of the hydrochloric acid, but our results appearing in the second part of this paper show that these cells do not normally contain the hydrochloric acid itself.

Miss Fitzgerald ('10) employed the Prussian blue reaction and obtained very definite results which must be carefully considered in the decision of this question. She found the Prussian blue deposited in the canaliculi of some parietal cells. It will be worth while to examine this reaction and the varying results which have been obtained with it.

The Prussian Blue Reaction.

This was first employed by Claude Bernard ('59) in his classic experiment. A translation of his account is as follows: "In a rabbit which had eaten very little there was injected into the jugular vein a solution of lactate of iron and then a solution of prussiate of potassium; both solutions were warm. Three quarters of an hour afterward the animal was killed and at the autopsy it was impossible to demonstrate the blue color in the tissue of any organ. The urine, which was alkaline and cloudy, was not blue, although it contained both prussiate of potassium and the iron lactate, for it sufficed to add a few drops of hydrochloric or sulphuric acid to cause the blue color of Prussian blue to appear immediately. Upon opening the alimentary canal a blue color was found on the surface of the mucous membrane of the stomach and particularly on the part which corresponded to the lesser curvature of that organ. But this blue was quite superficial; the little deposits of Prussian blue were only on the surface of the mucous membrane, and a microscopic examination did not reveal any Prussian blue in the gastric glands."

Later, Claude Bernard ('77) said: "The acid of the gastric juice is formed only after the secretion of the juice, the glands secreting a liquid which breaks up into an acid fluid and another product as yet not definitely determined."

The results which we have to report indicate that the decision reached by the great French physiologist is correct.

The Prussian blue reaction was employed also by Lepine ('72) in dogs. He used potassium ferrocyanide with lactate of iron or sulphate of iron. He was unable to obtain Prussian blue in any cells of the gastric gland either by injection, maceration, or by passing the salts through a dialyzing membrane made of the gastric mucous membrane, although by the latter method he did obtain a little blue in a lymphatic space of the connective tissue between the glands. He concluded that the acid was not formed as such within the gland.

Sehrwald ('89) put pieces of the gastric mucous membrane into a solution of lactate of iron for one day and later into a solution of potassium ferricyanide. He believed that in this way he would obtain a deposit of Prussian blue at the seat of formation of the acid. His results were not definite, although he thought the parietal cells showed more blue than other parts of the mucous membrane. The experiment was repeated by Miss Fitzgerald, who found the results too indefinite to decide the question, and by ourselves with the same outcome.

Fitzgerald's Paper.

Miss Fitzgerald ('10) conducted very careful and elaborate experiments by this method and has obtained by far the most definite results. Her experiments and the results are set forth in the following table, which is copied from her paper:[1]

We would like to direct especial attention to a few facts reported n this table and in her paper. First, the plates accompanying her paper show very clearly the deposit of Prussian blue within the canaliculi and in other parts of some parietal cells and also in the interglandular blood and lymph vessels, and in wandering cells and leucocytes. Second, in some experiments the Prussian blue reaction was not obtained. Third, when it did appear it was obtained in only one part of the stomach, namely, that near the œsophagus and along the lesser curvature. This is not the part which contains parietal cells in greatest abundance. Fourth, even in this region only a few of the parietal cells showed the Prussian blue. Those of the deeper third of the gland tubules, that is, the third farthest from the free surface never contained it; and in that part of the gland tubule where it did appear it was found in only a fraction of the parietal cells, so that altogether she found it in only a small percentage of the total number of parietal cells of the stomach, and this notwithstanding the fact that during some part of the long time the experiments lasted all parts of the mucous membrane must have been in full digestive activity. Fifth, it appeared in other structures as well as in the parietal cells, namely, in the blood vessels, in the connective tissue spaces and lymphatic vessels, in wandering cells, and in leucocytes. Sixth, in two instances (rabbits 5 and 6) it did not appear in the canaliculi of the parietal cells but only at the surface of the latter remote from the lumen and next to the blood vessels.

Our own results have confirmed these facts entirely.

TABLE GIVING RESULTS OBTAINED BY INJECTING A SOLUTION CONTAINING EQUAL PARTS OF AN AQUEOUS SOLUTION OF 2.25 PER CENT. AMMONIUM FERRIC CITRATE AND OF 1.5 PER CENT. POTASSIUM FERROCYANIDE.[1]

Animal.	Mode and No. of Injections.	Amount In-jected. C.c.	No. of Hours Since First and Last Injections.		Condition.	Toxic Effect.	Prussian Blue Reaction—Spontaneous.			Ditto on Addition of 0.5 Per Cent. HCl.	
							Gastric Mucosa.				
			First.	Last.			Surface.	Interior.	Skin.	Urine.	Various Tissues.[2]
Rabbit (1)...	Intravenous, 1 Subcutaneous, 2	10–11	$35\frac{1}{4}$	2	Food given.	−	−	−	−	+	+
Rabbit (2)...	Subcutaneous, 8	37	30	$2\frac{1}{2}$	Food given.	−	+	+	+	+ (1st day) − (2d day)	+
Rabbit (3)...	Subcutaneous, 5	31–32	$25\frac{3}{4}$	$2\frac{1}{2}$	Starved 24 hrs.	− (1st day) + (2d day)	+	−	−	− (Retention of urine)	+
Rabbit (4)...	Intravenous, 2	17	$4\frac{1}{2}$	$2\frac{3}{4}$	Semi-fasting. (Starved 36 hrs. Food. Starved 24 hrs.)	−	−	−	−	+	+
Rabbit (5)...	Subcutaneous, 5	50	$6\frac{1}{2}$	1	No food eaten during expt.	+	+	+	−	+	+
Rabbit (6)...	Subcutaneous, 4	43–45	3	$\frac{3}{4}$–1	Food before exp.	−	+	+	−	+	+
Rabbit (7) (sod. ferrocyanide)	Subcutaneous, 4	40	$5\frac{1}{4}$	1	Semi-fasting. Food given after fast of 20 hrs. and during expt. Eaten well until after 4th injection.	+	−	−	−	+	+
Guinea-pig (1)	Subcutaneous, 2	22	$3\frac{3}{4}$	$1\frac{3}{4}$	Food given.	+	+	−	+	+	+
Guinea-pig (2)	Subcutaneous, 2	16	$5\frac{1}{4}$	3	....	+ (Polyuria)	+	−	−	+ − ?	+
Guinea-pig (3)	Subcutaneous, 2	19	$6\frac{3}{4}$	$4\frac{1}{2}$	....	+ (Polyuria)	+	−	+	+	+
Dog (1)......	Subcutaneous, 2	55	$3\frac{1}{4}$	$1\frac{1}{4}$	Starved 48 hrs. Food given 1 hr. before expt.	+ (Vomiting)	−	−	−	−	+
Dog (2)......	Subcutaneous, 2	42	$3\frac{1}{4}$	..	....	+ (Comatose)	+	−	+	+	+

+ means positive result. − means negative result.

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[1] M. P. Fitzgerald, 1910, *Proceed. Roy. Soc.*, B. 83.

[2] After their removal from the body, and subsequent exposure to the action of dilute hydrochloric acid, the Prussian blue reaction developed, at various times, in the following tissues: Bladder, kidney, liver, small and large intestines, ovary, fallopian tubes, uterus, vagina, and lymph gland of axillary region (dog). The kidney became quite blue in many cases, and under the microscope the uriniferous tubules presented a very striking appearance.

From these results Miss Fitzgerald concludes that the hydrochloric acid was formed as such in the parietal cells and secreted into the gland lumina. She says (p. 82): "The occurrence of the Prussian blue reaction in the canaliculi of the parietal cells of an animal injected with a solution of these two salts affords conclusive evidence of the presence of free acid within these structures."

The absence of the Prussian blue from most of the parietal cells might be regarded as evidence that only a few of these cells are engaged in the formation of hydrochloric acid, and that the greater part of them do not form it. She explains this absence from most of the parietal cells by saying that it may have been washed out of them, but surely in that case it would have appeared in them in some preparations. She explains the appearance of the reaction in the blood vessels and in the part of the parietal cell next to the blood vessel (and that part only) by the suggestion that under certain circumstances the acid may be secreted by the parietal cells into the blood stream instead of into the gland lumen. But the facts she reports are open to the other interpretation that the Prussian blue, or the salts forming it, may have been excreted from the blood stream into the parietal cell.

Our Own Experiments.

These were conducted upon rabbits, cats, dogs, a fowl, a snapping turtle, and several skates. Into these animals we injected solutions of sodium ferrocyanide (which we found less toxic than potassium ferrocyanide) and solutions of iron and ammonium citrate. We did not always use molecularly balanced solutions, because we found that the two salts were excreted with very different degrees of rapidity and by different ways. The injections were made subcutaneously or intravenously. In so far as our experiments repeated those of Miss Fitzgerald, they confirmed the results reported by her entirely, but we obtained also some additional results which have a very important bearing upon the conclusion which she drew.

By these experiments we sought to get answers to the following questions: First, is the Prussian blue precipitate produced in any place other than the gastric mucous membrane?

Prussian Blue in Places Other than Gastric Mucous Membrane. —Into the ear vein of a rabbit was injected 13 c.c. of a 10 per cent. solution of sodium ferrocyanide, and into the other ear vein 10 c.c. of a 25 per cent. solution of iron and ammonium citrate; both solutions were warm. They were perfectly fresh, having just been made. They did not give a precipitate of Prussian blue when diluted each with an equal quantity of water, one added to the other, and the mixture allowed to stand in a warm chamber at 37° C. for 28 hours. The rabbit showed some toxic effects during the injection, rallied temporarily, but died in 20 minutes. The stomach was opened at once. It was full of fresh food in an active state of digestion. Prussian blue appeared in all parts of it, although the lesser curvature seemed to have less of it than the rest of the stomach. Pieces of various tissues were fixed in absolute alcohol. The urine showed both salts present in abundance; the bile contained none of either. The saliva contained ferric citrate but no ferrocyanide. The heart's blood showed no blue on the addition of acid nor on the addition of acid with each one of the two salts. Therefore, it did not contain either salt in any quantity appreciable by this method. Paraffine sections were cut and they showed the Prussian blue reaction on the surface of the stomach; in the mucous membrane of the stomach appearing in the blood vessels, in the lymphatic spaces, in the epithelium between the foveolæ; in the connective tissue of the muscularis mucosæ and of the tunica muscularis of the stomach. It did not appear in the parietal cells nor in any other gland cells, nor in the gland lumen. It was found in many other tissues, namely: in the liver, appearing in the blood vessels, in the endothelial cells of Kupffer, and a little in the bile capillaries; in the spleen appearing in the blood vessels; in the blood vessels of the heart muscle. These were the only places in which we looked for it, but we found it in all of them.

This experiment was repeated, using smaller quantities of the salts injected subcutaneously on two successive days (a total of 1.6 grammes iron and ammonium citrate and 3.0 grammes sodium ferrocyanide well diluted). The rabbit was killed 30 minutes after the second injection; the tissues were fixed in formalin (neutralized with magnesium carbonate). The Prussian blue reaction was found in all places in which it was found in the first rabbit. In addition it was found in the villi of the duodenum appearing in the connective tissue near the free end of the villus, and also between the epithelial cells of the free end of the villus. The blood vessels of the gastric mucosa contained Prussian blue, but in much smaller quantities than did those of the spleen and liver. The parietal cells were practically free of it; there may have been a little, for three or four minute particles were found in the section, but it was impossible to say that these extremely minute particles were not on the surface of the cell instead of within it.

From these two experiments it is evident that the Prussian blue is precipitated in the blood stream when solutions of these salts are injected into it. It may also be precipitated or absorbed in various places, especially in the endothelial cells of Kupffer in the liver. They show that it may appear on the surface of the stomach when it is not in the parietal cells; that it appears in the blood vessels and lymphatic spaces before it appears in the parietal cells. Its appearance between the cells of the surface epithelium between adjacent foveolæ, and also in the interglandular blood vessels and lymphatic vessels beneath this epithelium suggests that the Prussian blue or some or all of the salts necessary for its formation may pass from the surface into the interglandular lymphatics. These results taken in conjunction with those reported by Miss Fitzgerald in her rabbits Nos. 5 and 6 suggest very strongly that the Prussian blue or the constituents forming it may pass from the blood vessels into the parietal cells instead of in the reverse direction as she supposed. In other rabbits we have frequently seen the Prussian blue in the blood vessels and in only those parts of a few parietal cells lying next to the blood vessels, just as she reports in her rabbits 5 and 6. We have never found it in the parietal cells without finding it also in the blood vessels, but in these two instances we found it in the blood vessels when it was absent from the parietal cells. When it was present on the surface of the stomach we found it uniformly present also between the cells of the interfoveolar surface epithelium and in the interglandular lymphatic vessels and blood vessels. We have frequently found it similarly in the tips of pyloric and duodenal villi, between the epithelial cells and in the subjacent connective tissue. Similar results were obtained in a fowl which had been given subcutaneously 13 c.c. of a 10 per cent. solution of sodium ferryocyanide and 13 c.c. of a 25 per cent. solution of iron and ammonium citrate, and two hours later intravenously 7 c.c. of the sodium ferrocyanide solution and 4.5 c.c. of the iron and ammonium citrate solution; both solutions were fresh and warm. The animal stood these injections very well, although they had some toxic effect. The fœcal passages were liquid and contained a little blue precipitate which became quite heavy and abundant on the addition of hydrochloric acid. The animal was killed in one hour by chloroform, and pieces of various tissues were fixed in absolute alcohol. When the proventriculus was opened a blue fluid welled out of the openings of the depressions which contain the compound glands; no blue color appeared elsewhere in this organ. Microscopic examination of paraffine sections showed Prussian blue on the surface of the epithelium lining the necks of the depressions of the surface epithelium into which the glands open and in the lumina of these depressions. It was not within the glands themselves. It was present between the epithelial cells of the region where the depression receiving the secretion of the glands opened on to the surface of the proventriculus and in the subjacent blood vessels and lymphatic spaces. It was abundant in the blood vessels and lymphatic spaces of the muscular coat of the stomach, and of the crop, and of the heart muscle, and of the muscular stomach (gizzard). It appeared also in the epithelium of the intestine and in the connective tissue and muscle of the cœcal diverticula. It was abundant in the liver appearing in the endothelial cells of Kupffer and in the blood vessels. It was absent from the breast muscle and its lymphatic and blood vessels.

These results, therefore, answer this first question in the affirmative. The Prussian blue reaction appears in many places besides the gastric mucous membrane. In most of them it seems probable that it takes place without the help of an acid. And they show that, if its occurrence on the surface of the gastric mucous membrane is due to the acid formed in the stomach then that acid is present on the surface while it is absent from parietal cells and from the gland lumen.

Effects of Injury on Amount of Prussian Blue Precipitate.— Second, is the amount of Prussian blue precipitate increased by mechanical injury of the mucous membrane? A small kitten was given by subcutaneous injection small doses of a mixture of solutions of potassium ferrocyanide and iron and ammonium citrate in molecular proportions. These injections were given three times a day for three days. On the third day the abdomen was opened, a small piece of mucous membrane was removed from the fundus region of the stomach, the wound in the mucous membrane was sutured with silk sutures and afterward the other coats of the stomach were united and the abdomen was closed. The operation was done with aseptic precautions. Five hours later the kitten was killed, pieces of tissue were fixed in alcohol and in neutral formalin (freshly distilled over potassium hydrate). In the immediate vicinity of the suture paraffine sections showed that the Prussion blue reaction occurred in great abundance in the blood vessels, in the lymphatic spaces and in the parietal cells. These preparations showed blue in a very large proportion of the parietal cells and even in those at the bottom of the tubules. It was especially abundant in the parietal cells of the necks of the glands. Many parietal cells were dead and thrown off into the gland lumen; in every instance these dead cells were filled with Prussian blue. In those cells which were still in their normal position many showed the Prussian blue in the canaliculi disposed in a manner very similar to that which Miss Fitzgerald has shown in her Plate VII., Figs. 6 and 8. As one proceeded in the study of these sections progressively farther from the site of injury the amount of Prussian blue and the number of parietal cells showing it progressively decreased, and in some sections of mucous membrane taken from parts of the stomach remote from the site of operation and apparently in a healthy condition, the parietal cells did not contain any blue at all.

In another cat a similar operation was performed on the gastric mucous membrane and during four successive days following solutions of potassium ferrocyanide and of iron and ammonium citrate in equal quantities were injected subcutaneously. On the fourth day the cat died, but the tissues were immediately fixed in neutral formalin. There was more Prussian blue in the immediate neighborhood of the sutures than in other parts of the mucous membrane.

These results answer the second question in the affirmative. And they show that dead parietal cells show more Prussian blue than living ones. The dead ones are always stained by it, whereas most of the living ones are not. This suggests that the death of the parietal cell or a lowering of its vitality may permit or facilitate the penetration of the cell by substances concerned in the Prussian blue reaction, and that dead cells become acid.

Effect of Poisons on Amount of Prussian Blue Precipitate.—Third, is the extent of the Prussian blue reaction increased by the injection of poisons into the wall of the stomach? We injected into the submucous tissue of the stomach exposed under aseptic precautions, solutions of phosphorus in olive oil, and solutions of moccasin venom in distilled water. The incisions in the abdominal wall were closed and salts of sodium ferrocyanide and iron and ammonium citrate injected subcutaneously for varying periods. The results were negative; we could not demonstrate any increase of the Prussian blue reaction in the area where these poisons were injected.

Effect of Restriction of Blood Supply on Amount of Prussian Blue Precipitate.—Fourth, is the amount of Prussian blue increased in areas of restricted blood supply?

Arteries and veins of various sizes were tied on the stomachs of different rabbits and cats. Solutions of sodium ferrocyanide and iron and ammonium citrate were injected subcutaneously during several days. The results answered this question in the negative. There was no increase of Prussian blue in the areas of restricted blood supply. In a few cases ulcers were produced and on the surface of these there was always a considerable deposit of Prussian blue. The cells on the surface of the ulcer belonging to various parts of the gastric glands and presumably dead or dying always showed a considerable deposit of Prussian blue in them.

Precipitates Do Not Back into Gland Lumina from the Surface.—

Fifth, is the occasional presence of Prussian blue in the gland lumen and in the canaliculi of the parietal cells due to backing up of the blue precipitate from the foveolæ or from the surface of the mucous membrane.?

In order to determine this question many experiments were made with Prussian blue, with carmine and with India ink. Hydrostatic pressure, dialysis, and positive pressure by a syringe piston against a piece of gastric mucous membrane tied over the end of the syringe were employed. The results were all negative, thus confirming those of Lepine ('72).

Prussian Blue Reaction in Animals which Have no Parietal Cells, but Secrete Acid.—Sixth, where does the Prussian blue reaction occur in animals which have no parietal cells but yet secrete acid?

The experiment above reported with the fowl showed that the Prussian blue occurred in the lumen of the depression receiving the secretion of the compound glands of the proventriculus. It was not found in the gland cells. It appeared between the cells of the surface epithelium immediately adjacent to the opening of the depressions. As above stated, it occurred also in many other places.

A snapping turtle was given one half gram each of sodium ferrocyanide and iron and ammonium citrate in dilute solution three times daily during four days; the solutions were given separately and subcutaneously in the inguinal region. Two small fish were shoved into the stomach with a glass rod and were found there later partially digested. On the fourth day the animal was killed and the stomach examined. A deposit of Prussian blue was found in every cell of the somewhat coarse foveolæ of the gastric gland. In these cells it occupied a definite position, the same in each cell. Each cell contained a mucous plug which occupied the half of the cell next the lumen, under it a small spherical mass of Prussian blue, and under that the nucleus. The mass of Prussian blue was nearly as large as the nucleus. Sections through these foveolæ stained with paracarmine or mucicarmine presented a very pretty appearance because of the extreme regularity of the position of the nuclei, the little masses of Prussian blue and the mucous plugs. There was more Prussian blue in the cells of the foveolar epithelium then anywhere alse. The amount in the cells of the necks of the gland was less and decreased toward the bottom of the gland tubule, although it appeared in nearly all the cells of the gland. It was abundant in the interglandular connective tissue, being in the blood and lymph vessels.

Skates experimented upon in the same way showed Prussian blue in the blood vessels and lymphatics of the wall of the pharynx and stomach. The glands of the gastric mucous membrane showed the reaction in small quantities in the gland lumina and in a few of the cells. The cells showing the reaction were more numerous in the part of the gland near the free surface than in its deeper portions. The surface epithelium of the stomach and of the intestines two inches beyond the pylorus and of the large intestine contained small quantities of Prussian blue.

Summary of the Results Obtained by the Prussian Blue Reaction and their Significance.

From the facts reported by Miss Fitzgerald and those which our own experiments have added, it seems clear that the occurrence of the Prussian blue reaction does not necessarily indicate the formation of free mineral acid under normal conditions in the places in which it is found. It appears in many places where it could hardly have been due to the presence of acid—in the blood, lymph, liver, spleen, intestine, heart muscle, etc. In these places its presence must be due to something else.

It may be due to fatty acids, which bring about the precipitation of Prussian blue from solutions of the salts used in these experiments in proportion to the amount of the acid present. It may be due to the withdrawal of the ammonium citrate by more rapid diffusion or by the involvement of the ammonium in the metabolic processes of the tissues. Nencki and Pawlow ('96) have shown that the gastric mucous membrane normally contains an extraordinarily large amount of ammonia. Ferric citrate and sodium ferrocyanide solutions give when mixed an immediate precipitate of Prussian blue even in the absence of any acid. It may be due to the death or reduced vitality of cells, permitting the entrance of salts which would not have entered living or healthy cells. It may be that the cells which show the reaction are just those which are poisoned by the salts used. The iron and ammonium citrate solution injected repeatedly during several days caused the death of one kitten. Therefore this salt has a serious toxic action. It may be that the interaction chemically of these two salts with the contents of certain cells may sometimes permit the liberation of an acid in them, when no acid would have been produced in the absence of the complex chemical state which exists when they are present. It may have been absorbed from the surface of the mucous membrane. Its presence between the epithelial cells and in underlying lymphatic vessels of the stomach and intestine suggest this possibility.

Since the Prussian blue may be precipitated in so many places, the fact that it is sometimes precipitated in the canaliculi of a few parietal cells in a relatively small part of the stomach perhaps in an abnormal condition at the time does not necessarily prove that free hydrochloric acid is formed under normal conditions in the parietal cells of the stomach as a whole. The failure to get any reaction in the stomach in some experiments, the small number of parietal cells in which it ever appears, its occurrence in other tissues and in the blood vessels and lymph vessels before it appears in the parietal cells at all, the fact that several factors other than the presence of free mineral acid may cause the precipitate to form,—all these things show that it would not be right to conclude from the evidence which the Prussian blue reaction affords that free hydrochloric acid is formed in the parietal cells. Much less could one reach this conclusion from any other evidence that has been adduced, for all other evidence is much less definite than this. And this failure to show clearly that free hydrochloric acid is formed in the parietal cells becomes quite clear when it appears, as we shall show in the following part of this paper, that the contents of the canaliculi of these cells are alkaline and those of the gland lumina are not acid when free acid is being produced by the mucous membrane.

EXPERIMENTS WITH INDICATORS: THE REACTION OF THE SECRETION WITHIN THE LUMEN OF THE ACTIVE FUNDUS GLAND, AND WITHIN THE INTRACELLULAR CANALICULI OF THE PARIETAL CELLS.

It is obvious from the observations of Fitzgerald and ourselves reported in the preceding section that the Prussian blue reaction is not a trustworthy indication of the place of formation of the hydrochloric acid of the gastric juice, and that we must look to other methods for a solution of this problem. One naturally turns to the chemical indicators for this purpose. The results obtained by means of these substances by previous investigators who have employed them have been uniformly unsatisfactory and unconvincing. The most definite results obtained by these methods are those reported by Fränkel ('91) who used neutral sodium rosanilinsulphonate as an indicator, and by Edinger ('79), who employed a solution of sodium alizarin.

Rosanilin sulphonic acid possesses the property of forming with sodium hydroxide acid salts which form red solutions (acid fuchsin), and neutral salts whose solutions are colorless. The addition of small quantities of acid to solutions of the neutral salt results in the production of the red colored acid salt. Accordingly, Fränkel injected into the jugular veins of dogs 50–100 c.c. of a 5 per cent. solution of the neutral sodium rosanilin sulphonate. As a result of this proceeding he found the entire mucous membrane of the stomach, including the pyloric mucous membrane, stained brilliant red. Teasing portions of the mucous membrane in distilled water he found that both parietal and chief cells were stained in the fundus glands, and that the cells of the pyloric glands were also stained, while the cylindrical cells of the surface were unstained. He could see no difference in the intensity of the stain in the two types of cells. The same experiment was also performed on rabbits, but in this case he found that the color was not uniformly distributed throughout the mucous membrane, and the pyloric mucosa showed only a few slightly red spots.

From these experiments Fränkel concludes: that the mucous membrane of the stomach has an acid reaction; that the acid is formed in the parenchyma cells; and that it can always be demonstrated in them. Regarding the acid reaction obtained by this method in the pyloric region he does not venture an interpretation.

The results obtained by Edinger by means of the sodium alizarin reaction were similar to those of Fränkel. Sodium alizarin, as Edinger pointed out is, in neutral solutions, of a deep purple red color, while the addition of an acid results in the precipitation of the alizarin as a flocculent yellow precipitate. Edinger prepared the solution by adding alizarin in excess to a 10 per cent. solution of sodium hydroxide. Then the solution was filtered. 25–100 c.c. of this solution were injected into the jugular veins of rabbits and dogs. In a rabbit he found after this injection the stomach spotted red-violet and yellow, the latter being more general in the region of the greater curvature, though the pyloric mucous membrane was also yellow. He concludes that the glands of the rabbit's stomach are not all in activity at the same time, and that both the fundus and pyloric mucous membranes react acid. In dogs, after similar treatment, the whole mucous membrane of the stomach, including that of the pyloric region, was yellow. Sections of the mucous membrane showed that the yellow color was to be found at all levels, but the intensity of the stain was too slight to permit of the recognition of the stain in particular cells. The pancreas also gave an acid reaction.

Experiments with tropeolin, congo red, litmus, phenolphthalein, and other indicators in common use, have been without result or, at the most, have only indicated what was known from examination of the secretion, namely, that the contents of the stomach were acid.

It is obvious from the foregoing statements that the results of the experiments of Edinger and Fränkel with sodium alizarin and sodium rosanilin sulphonate were not in accord with what had been previously determined concerning the place of formation of hydrochloric acid in the stomach, inasmuch as they indicated the formation of acid in the pyloric mucous membrane which had previously been shown by Heidenhain ('70) and Klemensiewicz ('75) to secrete an alkaline fluid. Moreover, neither of these experiments gave any clear indication of the source of the hydrochloric acid.

It is also clear that in order to solve this problem by the use of a chemical indicator the substance employed, in addition to being an indicator of acidity or alkalinity, must have the properties of a vital stain, that is to say, the cells of the gastric glands must be freely permeable to it and it must have a special affinity for constituents of the gastric secretion in the glands, or the distribution coefficient must favor its concentration in this secretion in sufficient amounts to give a distinct color reaction. These conditions we have found to be fulfilled by neutral red and by a number of dyes belonging to the naphtol blue series including nile blue, and the various cyanamins discovered by Witt ('90).

Our first successful experiments in staining specifically the secretion in the parietal cells and in the lumina of the gastric glands were obtained with Grübler's napthalin blue R crystals (a trade name for naphtol blue). Solutions of this dye in normal salt solution, injected into the blood vessels of the recently killed animal, were found to stain the secretion in the canaliculi of the parietal cells and in the lumina of the gland tubules of the fundus region a distinct red color, while the cells of the foveola and the mucus on the free surface were stained a deep blue.

Tests of the solution of naphtalin blue afforded no explanation of this result, inasmuch as addition of acid produced no change in the color of the solution, and addition of sodium hydrate gave a green color. Accordingly, it seemed probable that the reaction observed was due either to another dye present in the naphtalin blue as an impurity, or to a new dye synthesized during the process of staining.

After consideration of the commercial process for the manufacture of the naphtol blues it seemed probable, in view of the fact that dimethylparaphenylendiamin is a biproduct of the synthesis of naphtol blue from nitrosodimethylanilin and B naphtol, that the dye on which this reaction depended would prove to be cyanamin, which, according to Witt, is formed when the mixture of naphtol blue and dimethylparaphenylendiamin resulting from the synthesis above mentioned is boiled for a time with an alcoholic solution of potassium hydroxide. Accordingly, cyanamin chloride was prepared by the process described by Witt, and its solutions tested on the gastric mucous membrane.

Cyanamin, according to Witt, possesses two basic groups, one molecule of the base combining with two molecules of hydrochloric acid to form a bichloride which is soluble in water with a deep blue color. On dilution of this solution the compound is broken up into a monochloride insoluble in water, which deposits as a reddish violet precipitate, and hydrochloric acid, which remains in solution. On the addition of alkalies the solution changes to a red color and after a short time the base settles out as a red flocculent precipitate.

On account of the formation of a monochloride intermediate in color between the red base and the blue bichloride it is apparent that as an indicator of reaction cyanamin does not approach in delicacy of response the more commonly used chemical indicators. But when we consider the relatively high content of hydrochloric acid in the gastric juice (as high as .5822 per cent. according to Rosemann ('07)) this is of little importance, for we have found that in dilute solutions of the dye a concentration of .0009 per cent. of hydrochloric acid, or approximately 1/600 of the concentration in the gastric juice, is sufficient to abolish all trace of red color. Furthermore, if Pawlow's idea is true that the native secretion has a constant acidity, and that the variations in acidity of the secretion from a gastric fistula are due to different degrees of neutralisation by the alkaline mucous secretion of the surface epithelium, then we might expect a maximum acidity in the gland lumen assuming that the hydrochloric acid is secreted as such by the cells. It follows therefore that if cyanamin stains the gastric secretion in the glands it will stain it blue wherever the acid is produced.

The method of applying the cyanamin is as follows: A fresh concentrated solution of the bichloride in normal sodium chloride solution is prepared; the animal is killed by a blow on the head, or by bleeding from the carotid, and the stomach exposed as rapidly as possible; a small piece of the mucous membrane is cut out with scissors, rinsed in normal salt solution, and placed in the solution of the dye. A few minutes' immersion suffices to accomplish the staining. When this is complete the piece of mucous membrane is placed on a slide with the mucous surface downwards and observed with a low power of the microscope.

If the staining has progressed far enough the edge of the preparation may be teased with needles and the superficial glands which alone are stained so isolated, when a cover glass is applied and the preparation studied by high power objectives. For these experiments we have used rabbits, guinea pigs, cats, and dogs.

In such preparations certain cells scattered throughout the glands promptly stain blue, the blue color affecting not only the protoplasm but the nucleus. These belong to both classes of cells constituting the glands and are interpreted by us as dead cells. In addition the small cells, first described by R. Heidenhain, which occur in small numbers scattered among the other epithelial cells of the gland, and the nature of which is still obscure, stain blue, but in this case the blue stain is confined to the granules with which the protoplasm of these cells is studded, the nucleus remaining unstained. Certain glands on the very edge of the preparation may stain bluish red, these being for the most part glands which have been actually injured in making the preparations.

In the uninjured glands reached by the dye, on the contrary, a uniform and characteristic reaction is obtained. With the exception of the dead cells and the small cells of Heidenhain mentioned above, the dye is entirely confined to the secretion in the lumina of the glands and their various diverticula, including the whole basketwork of canaliculi in the parietal cells—all of which was intensely stained. Moreover, in no place in this system of gland tubules below the level of the gastric foveolæ was the blue color of the acid solutions of the dye obtained. On the contrary the secretion contained in the canaliculi of the parietal cells was a distinct red like that displayed by the dye in alkaline solutions, while the secretion in the lumen of the gland was a bluish red. The short canaliculi connecting the parietal cell system of intracellular channels with the main lumen of the gland showed a color shading from the red of the content of the latter to the bluish red of the contents of the gland lumen. At the level of the bottoms of the foveolæ the color of the secretion changed rapidly to the pure blue of the acid solutions of cyanamin, and the cylindrical cells of the surface and of the foveolæ stained the acid color also.

Inasmuch as the results just described indicated that in no part of the gland system below the foveolar level did the secretion of the gastric gland cells have an acid reaction, and that the secretory contents of the parietal cells were even alkaline in reaction it was important to test the behavior in different states of physiological activity of these glands towards solutions of the dye. These experiments were performed on dogs, animals being kept without food for twenty-four hours, and compared, as regards the reaction with cyanamin chloride, with other animals at different intervals after feeding. These experiments showed that the resting gastric glands gave no reaction with cyanamin, while glands taken from active stomachs fifteen or more minutes after secretion gave the pronounced and characteristic reaction described above. Accordingly, the alkaline reaction of the contents of the canaliculi of the parietal cells, and the non-acid reaction of the contents of the lumen of the gland proper, are not the reactions of resting glands, but only of active glands from a stomach which is forming an acid secretion.

The amount of cyanamin chloride at our disposal did not permit our testing its action on the stomach when injected into the living animal intravenously, or by injection immediately after death of solutions through the blood vessels. Naphtol blue, however, apparently owes its properties in this connection to admixture of cyanamin, or to synthesis of the latter during the process of staining, for the reaction which it gives is exactly that of the pure cyanamin solutions, and we have been able to separate from the commercial zinc naphtol blue double chloride small quantities of cyanamin. Naphtol blue dissolved in normal salt solution injected from the aorta in a rabbit killed shortly after feeding will produce this reaction in every gland of the fundus region of the stomach. It is difficult, however, by this method, to secure a staining of the entire gland, for reasons which a consideration of the blood supply of the mucous membrane will make apparent. The bases of the glands stain well, but it is difficult to secure a staining of the upper portions of the glands. Preparations made in this way give, however, the most remarkable demonstration of the canalicular system of the glands that we have ever seen, resembling except for the color a perfect silver chromate impregnation of this system. We have also obtained a feeble reaction by means of intravenous injection of solutions of naphtol blue.

CONFIRMATORY TESTS.

In view of the results obtained with cyanamin chloride it seemed probable that other dyes closely related to this substance would give similar reactions, and accordingly we prepared by acting on naphtol blue with anilin, according to the method described by Nietzki and Bossi ('92), the closely related dye named by the former phenylated nile blue. Solutions of this dye gave by far the most striking results obtained inasmuch as the intensely red base was precipitated in the canaliculi of the parietal cell, while the secretion in the gland lumen was stained a bluish red color. Similar results were obtained with solutions of nile blue sulphate, but a less pronounced reaction was obtained, the content of the parietal cells staining in this case bluish red, that of the lumina of the glands blue.

Neutral red, which has been highly commended by Ehrlich as an indicator for biological studies, next suggested itself in this connection for we had long known that it stained the secretion in the gastric glands and in the parietal cells. This dye may be used like cyanamin by immersing the fresh mucous membrane in a 1 in 10,000 solution in normal salt solution or by injecting such a solution of the dye through the blood vessels. In neutral solutions neutral red possesses a reddish color with a suggestion of orange. Alkaline solutions precipitate the base in the form of a yellow precipitate while acid solutions produce a crimson color. This dye therefore is capable of indicating either acidity, alkalinity or neutrality. In preparations made as indicated above of the fresh actively secreting fundus mucous membrane of the stomach neutral red promptly stains the secretion in the canaliculi of the parietal cells and in the main lumen of the gland. In the parietal cells the color assumed is the unmistakable yellow of the free base, in the lumen of the gland the color approaches more closely to the neutral tint, while the short diverticula of the lumina which connect the parietal cell with the lumen are of an intermediate tint. The whole system, however, is without question on the alkaline side of the reaction with neutral red. At the bottom of the foveolæ the alkaline reaction gives way to the crimson acid color which is exhibited by the whole foveola and by the surface. The foveolar epithelium also stains the crimson acid tint. Neutral red stains the dead cells deep red, and also the granules of the small cells of Heidenhain referred to above.

Thus the consistent results of four separate methods show that the hydrochloric acid is not free as such in the gland, and that the contents of the canaliculi of the parietal cell contrary to expectation are alkaline in reaction. The question naturally arises, then, where is the acid of the gastric juice formed and what are the factors concerned in its formation? Without doubt, our reactions with the dyes of the cyanamin series indicate that the hydrochloric acid of the gastric juice is set free in the foveola, possibly also on the free surface of the mucous membrane. As to the source of the chlorine concerned in the formation of hydrochloric acid of the stomach the experiments of Greenwood ('85), Macallum ('08), and Fitzgerald ('10) seem to be conclusive. Greenwood showed that in preparations of the mucous membrane of the stomach made with silver nitrate, and then reduced in the light, the parietal cells stained much more strongly with the silver deposit than the other epithelial elements. In his studies of the silver reaction for chlorides Macallum showed that only chlorides, phosphates, and carbonates, of silver gave this reduction reaction, and devised a method by means of which the phosphates and carbonates could be excluded and only chlorides exhibited. This method consisted in using for the reaction a solution of silver nitrate containing nitric acid in which the phosphates and carbonate of silver are soluble. By this means he demonstrated that the parietal cells of the stomach were rich in chlorides. This result has recently been confirmed by Miss Fitzgerald, who found that the reaction was obtained not only in the body of the parietal cell but also in the intracellular channels.

This being the case, in view of the fact that the secretion of the parietal cells is alkaline while in the cells themselves, and that the secretion of the whole gland while contained in the gland lumen is very nearly neutral as shown by the neutral red and cyanamin reactions, it seems probable that the chlorine is secreted by the parietal cells in the form of a chloride of an organic base, and that the hydrochloric acid is only set free after this secretion is poured out of the gland into the foveola. As to the nature of this base, there are some facts which suggest the probability that it is protein in nature. Stöhr's ('82) description of the parietal cells in man indicates clearly that he perceived a coagulated substance in the canals which connect these cells with the lumen of the gland, and Revell has succeeded in staining the content of the intracellular canals of the parietal cells with carmin solutions in material fixed in an alcohol bichromate sublimate mixture.

THE CONSISTENCE OF THE SECRETION IN THE GLAND LUMEN.

The fact that we were able to stain the secretion of the gastric glands while still contained in the gland has enabled us to study certain properties of this secretion. In the actively secreting rabbit stomach the lumen of the gland is widened by the accumulation of the secretion, and, by pressure on the cover glass, or by teasing, it is possible to expel the secretion from the gland, or to liberate it in the salt solution used for mounting and thus to learn something about the change in concentration of the secretion which takes place as it proceeds towards the surface of the mucous membrane. The assumption which is generally made that the secretion is formed by the glands in the same concentration as it presents when it emerges from the openings of the foveolæ, would lead one to suppose that the secretion in the gland would be a limpid solution, which would flow easily from the gland, and would mix readily with salt solution. This, however, proved not to be the case. When water from the surrounding salt solution enters the gland lumen the column of secretion breaks up into round droplets which maintain their individuality for several minutes. Similarly, when secretion is expressed from the gland lumen into the surrounding solution it collects around the mouth of the gland in large spherical droplets which slowly dissolve, the red reaction also at the same time slowly changing to the blue acid reaction, if the secretion has been stained with cyanamin. From these observations we are obliged to conclude that the secretion formed in the gland possesses a relatively high content of solids, and that the bulk of the water found in the gastric secretion is added at the level of the glandular foveolæ.

OTHER CONSIDERATIONS.

Since it is apparent that the contents of the gastric glands proper when in a state of normal activity are not acid in reaction, and may even be alkaline, it follows that the ferment of the same secretion in the gland lumen is probably not in an active form, since, as is well known, pepsin is destroyed by alkalies while pepsinogen is not affected. Hence, the failure of the secretion to attack the cells themselves requires no further explanation in the case of the gastric glands than in the case of the pancreas, since in neither case does the activated ferment come in immediate contact with the parenchyma cells. This being the case it is pertinent to enquire whether under any conditions the secretion within the gland may become acid in reaction, for, in this event it is probable that the ferment would be activated and as happens under similar conditions in the pancreas the adjacent parenchyma cells would be attacked. This possibility is suggested by certain results obtained with the Claude Bernard reaction, where a reaction was obtained in the neighborhood of recent injuries to the mucous membrane far down the lumen of the gland, though the rest of the mucous membrane showed no reaction in the glands. We have as yet not had the opportunity to test this question by means of the cyanamin and neutral red reactions, but hope to report on this matter in the near future.

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THE PHYLOGENY OF THE NEMOCERA, WITH NOTES ON THE LEG BRISTLES, HAIRS AND CERTAIN MOUTH GLANDS OF DIPTERA.[1]

W. WESCHÈ, F.R.M.S.

Professor Williston has published a paper[2] on the antennæ of Diptera which is not only a remarkable analysis of these organs, but one showing an encyclopædic knowledge. His deductions combined with his observations on other characters seem to me of great weight and importance, and may lead to a more exact classification of the order.

On page 326 of the cited paper, in commenting on the number of antennal joints in the different families of Diptera, he says: "We are at once struck with the predominance of five groups having a maximum normal number of sixteen, fifteen, ten, six and five. And I venture to suggest that these five groups represent in the main five different divergent phyla of Diptera." These groups are:

Group 1.—Families having from twelve to sixteen joints: Tipulidæ, Cecidomyidæ, Psychodidæ, Mycetophilidæ, Pachyneurinæ, Rhyphidæ.

Group 2.—Families with from six to fifteen joints: Dixidæ, Culicidæ, Blepharoceridæ, Chironomidæ.

Group 3.—Families with from seven to ten joints: Scatopsinæ, Simulidæ, Xylophaginæ, Stratiomyidæ, Acanthomeridæ, Tabanidæ.

Group 4.—Families with from three to six joints: Nemistrinidæ, Lonchopteridæ, Phoridæ, Cyclorrhapha.

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[1] The MS. of the present article was sent to me some time before his death by the late Mr. Weschè, with a request for comment and criticisms. In editing the paper, which Mr. Weschè had not quite completed, I have made no changes whatever except verbal ones, and have omitted only a few immaterial parts. Most of his conclusions seem well taken, and it is to be regretted that the author could not have been spared to continue his researches along the fruitful lines that he had begun.—S. W. WILLISTON.

[2] BIOLOGICAL BULLETIN, XIII., p. 324, 1907.

Group 5.—Families with from three to five joints: Mydaidæ, Apioceridæ, Asilidæ, Therevidæ, Bombylidæ, Dolichopodidæ, Empidæ.

The Orphnephilidæ (11, 12), Bibionidæ (8–12), Leptinæ (3–8) and Scenopinidæ (3) fill in gaps between the chief groups. But it is obvious that the author was not dogmatic in the formulation of these groups, as later it is stated (p. 330) that "the antennæ, taken separately, are only partial evidences of relationship. They must be correlated with all other organs of the body, and must harmonize with theories based upon other organs." Carrying out this idea, and quite agreeing that no single character can be relied upon, I have endeavored to test the validity of these phyla by other structures, for the most part microscopic, such as the eyes, the trophi, and the genitalia; and I have embodied the results of my studies in a series of tables.

These tables have been made to show the dominant characters of the families; every large family has numerous exceptions and specializations, as, for instance, the labium in the Dolichopodidæ is nearly always short, though we know that it is long in *Orthochile*, and longer than normal in *Gymnopterus*. In speaking of dominant characters I must guard myself by saying that my cabinet of dissections is composed mostly of the commonest and most widely distributed forms, selected where the material for dissection was most abundant; there is, hence, probably a margin of error.

The tabulated observations are mostly contained in four papers previously published by myself: "The Mouthparts of the Nemocera," 1904, with additions and corrections (1909), (*Journal of the Royal Microscopical Society*); "The Genitalia of the Sexes in Diptera" (*Trans. Linn. Soc.*, London, 1906), and "The Structure of the Surface and the Sexual Characters of the Eyes of Diptera" (*Journal Queckett Club*, 1909). Many additional facts since observed have been incorporated in the tables.

The following explanations will be necessary for a full comprehension of these tables. The trophi are fully analyzed. Their most important parts, from the point of view of phylogeny, seem to be the tracheæ of the paraglossæ, the mentum, the palpi, and the pharyngeal pump. The tables of the genitalia give the characters of the interior and more invisible parts, which I have formulated in a previous paper.[1] They will be described as of certain types:

Type 1.—A long flagelliform tube, as in *Tipula*, or an approximation to that type.

Type 2.—A prominent chitinous bulb, with lateral processes, as in *Ptychoptera*.

Type 3.—A low membranous process supported by chitinous levers, as in *Gymnoplista* and *Culex*.

In the second case the nature of the ancillary claspers are given (*a*) as simple hooks, as in many Muscidæ, (*b*) jointed, as in the Tabanidæ, (*c*) or a simple unsegmented cercus-like appearance, as in the Bibionidæ. Three types of ovipositor are differentiated:

Type 1.—Telescopic or protrusile, as in *Calliphora* or *Dolichopus*.

Type 2.—Nontelescopic, as in *Tipula*, where it can scarcely be said to exist.

Type 3.—Short segmented, such as is found in the Empidæ.

Further the appendages of the egg-guide are tabluated, as (*a*) cercus-like, as in *Bibio* or *Musca;* (*b*) uncinate, as in *Tipula* or many Muscidæ; (*c*) styliform, as in *Psychoda* or *Pipunculus*, where it is probably a fusion of the pair of forceps.

The number of receptacula is noted. The types of penis and ovipositor seem to me the more important characters. Among the characters of the eyes, holopticism, dichopticism, and the greater width of the female front are given; and the absence or presence of ocelli is also noted.

It may be stated that in certain families the compound eyes are chitinous plates pierced by circular facets or lenses; this opaque structure is much reduced in other families till only strips of chitin separate the hexagonal facets. Finally in specialized groups all traces of opaque structure are lost.

These tables may be examined in two ways. The most important characters may be noted and the tables consulted to see how far they are in agreement; or the characters of the group may be added up and averaged. The latter method shows that

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[1] "Notes on the Value of the Genitalia in Phylogeny." *Trans. Entom. Soc. London*, 1908.

TABLE I.

MOUTH CHARACTERS OF THE NEMOCERA.

	Group.	Labium.				Tracheæ.		Mentum.				Labrum.				Hypopharynx.			Palpi.					Sense Organs.			Mandibles.				Maxillæ.			Pharyngeal Pump.			
	Williston's Phyla.	Short.	Long.	Membranous.	Chitinous.	Present.	Absent.	Undeveloped.	Developed.	Extremely Developed.	Median Suture.	Strong.	Weak.	Ciliated.	Vestigial.	Ciliated.	Weak.	Vestigial.	Maxillary.	Labial.	4 Joints.	2 Joints.	1 Joint.	Absent.	Present.	Peculiar.	Fully Developed.	Vestigial.	In Ventral Side of Labium.	Fused into Dorsal Side of Labium.	Laciniæ Present.	Stipites and Cardines Present.	Vestigial.	3 Plates.	2 Plates.	Vestigial.	Absent.
Cecidomyidæ	I	*		*			*	*				*				*			*		*			*					*			*					
Mycetophilidæ	I	*		*		*	*	*				*				*			*		*				*				*		*	*				?	
Bibionidæ: *Bibio*	0	*		*			*	*				*		*		*				*	*				*				*			*					*
Bibionidæ: *Dilophus*	0		*	*			*	*					*			*	*			*	*				*				*[1]				*				*
Bibionidæ: *Scatopse*	3	*		*			*	*				*				*				*			*		*				*				*		*		
Simulidæ	3	*		*			*		?			*				*			*		*				*	*	*				*	*		*[4]	*		
Chironomidæ	2	*		*			?	*					*			*			*		*			*					*			*					*
Ceratopogon	2		*	*			*		?				*			*			*		*			*	*		?[2]		*		*	*			*		
Psychodidæ	I	*		*			*	*					*			*			*		*			*					*		*	*			*		
Culicidæ: *Corethra*	2	*					*		*		?		*			*			*		*			*					*		*	*		?			
Culicidæ: *Mochlonyx*	2	*			?		*		*		?		*			*			*		*			*					*		*	*		*			
Culicidæ: *Culex*	2		*		*	*	*		*	*			*			?			*		?	?		*			♂				*	*		*			
Ptychoptera	I	*		*				?					*					*	*		*			*					*				*[3]		*		
Tipulidæ	I	*		*		*		*					*			*	*		*		*			*					*				*		*		
Rhyphidæ	I	*		*			*		*		*	*		*		*[5]			*		*				*	*			*		*	*			*		

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[1] Only a remnant of the fused parts.

[2] In one species.

[3] Fused together and in the same condition as the other Tipulidæ.

[4] This clearly shows as three in one preparation. I am not certain of two.

[5] Very vestigial cilia.

TABLE II.

CHARACTERS OF MOUTH IN BRACHYCERA AND CYCLORRAPHA.

	Group.	Labium.				Tracheæ.		Mentum.				Labrum.				Hypopharynx.				Palpi.					Sense Organs.			Mandibles.				Maxillæ.			Pharyngeal Pump.			
	Williston's Phyla.	Short.	Long.	Membranous.	Chitinous.	Present.	Absent.	Undeveloped.	Developed.	Extremely Developed.	Median Suture.	Strong.	Weak.	Ciliated.	Vestigial.	Ciliated.	Weak Unciliated.	Strong Unciliated.	Vestigial.	Maxillary.	Labial.	4 Joints.	2 Joints.	1 Joint.	Absent.	Present.	Peculiar.	Fully Developed.	Vestigial.	In Ventral Side of Labium.	Fused into Dorsal Side of Labium.	Laciniæ Present.	Stipites and Cardines Present.	Vestigial.	3 Plates.	2 Plates.	Vestigial.	Absent.
Asilidæ	5	*			*		*		*	*		*				*				*			*		*			*				*	*			*		
Empidæ	5		*	*	*[1]	*	[1]		*		*	*						*		*				*	*					*		*	*			*		
Dolichopodidæ	5	*		*		*[2]			*		*	*		*				*			*			*	*					*			*			*		
Phoridæ	4	*		*		*			*		*	*						*			*		*[3]	*	*[4]	*				*			*[5]	*				*
Lonchopteridæ	4	*		*		*			*		*		*					*		*				*	*					*		*	*					*
Leptidæ	0	*		*		*		*[6]	*		*	*					*	*		*				*	*					*		*	*					*
Stratiomyidæ	3	*		*		*			*		*	*					*			*			*			*				*		*	*					*
Tabandidæ	3		*	*		*		*	*[7]			*						*		*			*		*		*[9]	*	♂			*	*				*[8]	
Bombylidæ	5		*	*	*	*			*		*	*						*		*				*		*[10]				*		*	*					?
Cyrtidæ	0		*[11]							*																												
Platypezidæ	4	*		*		*			*		*		*				*			*				*	*					*		*	*[12]					*
Pipunculidæ	4	*		*		*			*		*		*				*			*	*?			*		*				*	*?		*					*
Syrphidæ	4		*	*		*			*				*				*			*				*	*						*	*	*					*
Conopidæ	4		*		*	*			*	*			*		*		*		*	*				*	*						*		*					*
Muscidæ	4	*	*	*	*	*	*		*	*		*	*		*		*	*	*		*			*	*						*	*[13]	*					*

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[1] In *Hybos*.

[2] Peculiar.

[3] In several species.

[4] Only in *Trineura*.

[5] In an atrophied condition in a few species.

[6] In *Leptis*.

[7] In *Hæmatopota* and other species of *Tabanus*.

[8] In *T. africanus* and *H. pluvialis*.

[9] In *Pangonia longirostris*.

[10] Small in *Comptosia occellata* New.

[11] Or aborted.

[12] Vestigial in several species.

[13] In some Acalyptrates.

the points of agreement are fairly strong in group 1, strong also in group 2, weak in groups 3 and 4, and fairly strong in group 5.

If the weak groups are examined anomalies will be seen in group 3; the Simulidæ seem out of place. I have lately found a structure in the palpi of *S. reptans* and *Rhyphus fenestralis* and *R. punctatus* ♀, which convince me of a near relationship and common ancestry of these two families. By adding *Simulium* to group 1 there results a larger proportion of similar characters.

TABLE III.

CHARACTERS OF THE GENITALIA IN THE NEMOCERA.

	Group.	Male.						Female.								
		Type of Penis.			Claspers Forcipes.			Ovipositor.			Appendages.			Receptacula.		
	Williston's Phyla.	Type 1.	Type 2.	Type 3.	Cercilike.	Simple Hamate.	Jointed Hamate.	Non-telescopic.	Short Telescopic.	Telescopic.	Cercilike.	Uncinate.	Styliform.	One.	Two.	Three.
Cecydomyidæ	1	*				*		*			*			*[1]		
Mycetophilidæ	1	*			*	*			*		*					
Bibionidæ	0	*			*			*			*					*
Scatopse	3	*				*		*			*			*		
Simulidæ	3	*			*			*			*			*		
Chironomidæ	2	*[2]				*		*			*				*[3]	
Psychodidæ	1	*				*		*					*			
Culicidæ	2		*			*		*				*				*
Ptychopteridæ	1			*		*		*				*				*
Erioptera	1							*				*				*
Tipulidæ	1	*				*		*				*				*
Rhyphidæ	1	*[4]			*			*			*			*		

[1] Where nothing is stated these organs are not chitinous and do not show in preparations.

[2] Approximates to that of the Rhyphidæ.

[3] 1–2 in *Ceratopogon*.

[4] Complicated and peculiar, but approximates.

Nor can I reconcile myself to the inclusion of the Scatopsinæ, though this subfamily, unlike the Simulidæ, will not fit well into group 1, notwithstanding that the genitalia show marked affinities with those of *Tipula*. The very marked specialization of the mouth structure and the three ocelli outweigh in importance the archaic eye structure, the bristle structure, especially that of the legs, and pharyngeal pump; I can not at present suggest any change in the position they now occupy among the Bibionidæ, though I consider this family the most specialized of the Nemocera.

TABLE IV.

CHARACTERS OF THE GENITALIA IN THE BRACHYCERA AND CYCLORRAPHA.

	Group.	Male.						Female.								
		Type of Penis.			Claspers.			Ovipositor.			Appendages.			Receptacula.		
	Williston's Phyla.	Type 1.	Type 2.	Type 3.	Cercilike	Simple Hamate.	Jointed Hamate.	Non-telescopic.	Short Telescopic.	Telscopic.	Cercilike.	Uncinate.	Styliform.	One.	Two.	Three or More.
Asilidæ	5	*				*		*			*	*			*	
Empidæ	5	*				*			*		*			*		
Dolichopodidæ	5	*				*				*		*		*[1]		
Phoridæ	4		*	*	*					*	*			*[1]		
Lonchopteridæ	4		?[2]	?	*			*			*				*[3]	
Leptidæ	0	*					*		*		*					*
Stratiomyidæ	3	*			*				*		*					*
Tabanidæ	3	*[4]					*	*			*					*
Bombylidæ	5	*			*			*			*					*
Cyrtidæ	0	*			*			*			*			?		
Platypezidæ	4	*			*	*			*		*				?	
Pipunculidæ	4	*				*		*					*			*
Syrphidæ	4			*	*	*				*	*	*				*
Conopidæ	4			*	*			*	*		*	*				*
Muscidæ	4			*		*		*	*	*	*	*	*	*	*	*

[1] Invisible in preparations.

[2] Indefinite, suggests affinities with *Dolichopus*.

[3] Transparent, only demonstratable by dissection.

[4] And theLeptidæ are nearer type 1 than 2 or 3. They only approximate.

If any reliance is to be placed on the genitalia, the Stratiomyidæ must belong in group 3, since the male type clearly connects the family with the Asilidæ, Empidæ and Dolichopodidæ. And the condition of the mentum sustains this view, though character of the leg pubescence is less decisive. The genitalia and venation of the Tabanidæ are so close that it seems impossible to separate the family; both find their place in group 5.

These points seem to show that group 3 is an artificial one; nor does group 4 inspire me with confidence, since I can not separate the Phoridæ and the Lonchopteridæ from the Asilidæ, Empididæ and Dolichopodidæ. Group 4 must be narrowed down to the Cyclorrhapha, and even here I think that I can trace the pedigree to group 5, if not to group 1.

I have now completed my analysis of the groups suggested by Williston from the antennal characters, so far as my imperfect data will permit, and I think that it shows that they furnish important hints as regards the phylogeny, particularly of the older flies, though the light they throw is intermittent and flickering. From their exposed position and frail structure the antennæ are subject, in the swiftest flying insects, to more than the usual risk of injury, which is possibly one reason for the extreme variation they show, ranging from three joints in the Scenopinidæ to thirty-nine in *Cerozodia*.

TABLE V.

STRUCTURE OF EYES IN NEMOCERA.

	Group.	Chitinous Structure.				Facets.			Pubescence.				Plates.[1]		Sexual Characters.				Ocelli.		
	Williston's Phyla.	Marked.	Lines Remaining.	Absent.	Double Eyes.	Circular.	Hexagons.	Two Sizes in ♂.	Much in ♂.	Less in ♀.	Equal ♂ ♀.	Absent.	Marked.	Simple.	Holoptic.	Dichoptic.	Equal.	Extreme Development.	Three.	Two.	Aborted.
Cecidomyidæ....	I	*				*						*						*		*[2]	*
Mycetophilidæ...	I	*				*					*		*			*			*	*	
Bibionidæ: *Bibio* ♂....	0		*		*		*	*	*					*	*				*		
Bibionidæ: *Bibio* ♀....	0			*			*					*		*	*				*		
Bibionidæ: *Dilophus* ♂.	0		*		*	*	*	*	*					*	*				*		
Bibionidæ: *Dilophus* ♀.	0		*			*				*				*	*	*			*		
Bibionidæ: *Scatopse* ♂ ♀	3	*				*					*	*	*			*			*		
Simulidæ.......	3	*					*	*				*	*			*					*
Chironomidæ....	2	*				*	*					*	*			*					*
Psychodidæ.....	I	*				*						*	*			*					*
Culicidæ: *Corethra* ..	2	*				*						*	*			*					*
Culicidæ: *Mochlonyx*	2		*			*						*	*			*					*
Culicidæ: *Culex*.....	2			*		*						*	*			*					*
Ptychoptera.....	I	*					*					*		*		*					*
Tipulidæ.......	I	*					*					*		*		*					*
Rhyphidæ......	I		*				*				*			*		*			*		

[1] *Chironomus.*

[2] Exceptions exist.

To recapitulate, I think that group I, with the inclusion of the Simulidæ, is a strong natural group, and even stronger tribe. Groups 3 and 4 fail altogether. Group 5 is fairly strong, but suggests the inclusion of the Stratiomyidæ, Xylophaginæ, Tabanidæ and Leptinæ, Lonchopteridæ and Phoridæ. As regards the Acanthomeridæ I have nothing to suggest.

TABLE VI.

STRUCTURE OF THE EYES IN BRACHYCERA AND CYCLORRHAPHA.

	Group.	Chitinous Structure.				Facets.			Pubescence.				Plates.		Sexual Characters.				Ocelli.		
	Williston's Phyla.	Marked.	Lines Remaining.	Absent.	Double Eyes.	Circular.	Hexagons.	Two Sizes in ♂.	Marked in ♂.	Less in ♀.	Equal ♂ ♀.	Absent.	Marked.	Simple.	Holoptic.	Dichoptic.	Equal.	Extreme Development.	Three.	Two.	Aborted.
Asilidæ	5	*					*	[1]			*	*		*		*			*		
Empidæ *Clinocera*	5	*					*				*			*		*			*		
Empidæ *Pachymera*	5		*				*					*		*		*			*		
Empidæ *Hybos*	5			*			*					*		*			*	*	*		
Dolichopodidæ	5	*	*				*[2]				*			*	*				*		
Phoridæ	4	*	*			*	*[2]				*			*		*			*		
Lonchopteridæ	4	*					*					*		*		*			*		
Leptidæ	0	*					*	*			*	*		*	*				*		
Stratiomyidæ	3	*					*	*			*	*		*	*	*			*		
Tabanidæ *Tabanus*	3	*					*	*	*	*				*	*						*
Tabanidæ *Chrysops*	3		*				*				*	*		*	*				*		
Tabanidæ *Hæmatopota*	3	*	♂	♂[3]			*	*	*	*				*	*						*
Tabanidæ *Pangonia*	3	*					*	*				*		*	*				*		
Bombylidæ	5	*					*				*			*	*				*		
Cyrtidæ	0										*	*		*					*		*
Oncodes	0	*					*					*		*			*	*		*	
Platypezidæ	4		*	*			*					*		*		*[4]			*		
Pipunculidæ	4			*			*					*		*	*			*	*		
Syrphidæ	4	*					*	*			*	*		*	*				*		
Conopidæ	4			*			*					*		*		*			*		*
Muscidæ	4			*			*		*	*	*	*		*	*	*			*		

[1] In *Leptogaster cylindrica* Deg. (exceptional) ♀.

[2] Often squares.

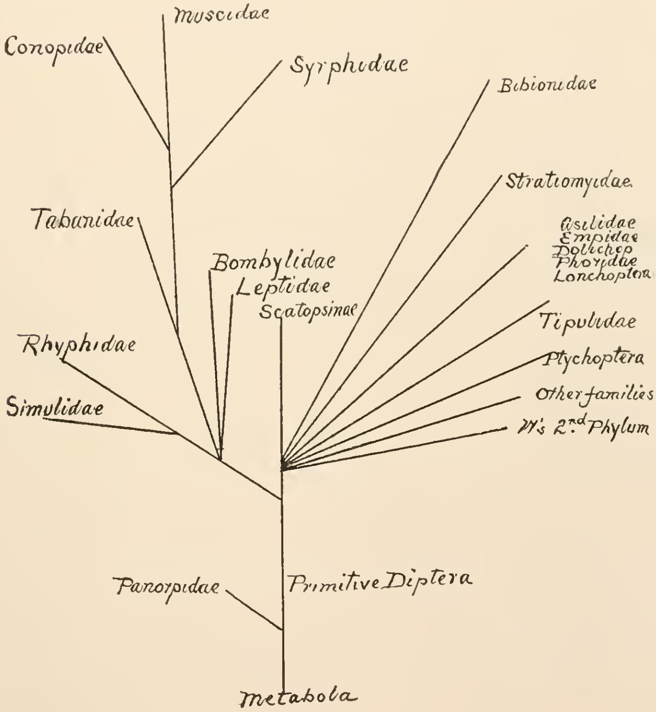
[3] The three stages have been found in the eyes of *H. pluvialis* in ♂.

[4] *Opetia* exceptional.

With regard to the phylogeny of the Cyclorrhapha I have endeavored to show by a comparison of the mouthparts and venation, to which I may add the eye structure and general morphology, that the Tabanidæ stand close to the ancestral forms of the Cyclorrhapha, though the Syrphidæ and Conopidæ branched off when the insects had complete mouthparts and ocelli, long before the Muscidæ became the specialized and dominant group that it now is.

Williston says (page 332) that "every family save the Tipulidæ is, I believe, absolutely excluded from immediate genetic relations with the Brachycera, because of the venation and antennæ." I do not know of any anatomical structure that militates against this view, and there are several that favor it; but I am strongly inclined to associate the Rhyphidæ with the Tipulidæ. The antennal characters and the venation are not insuperable objections; but I rely on the mouth, simple eyes and genitalia. The first of these characters is much less specialized than in *Tipula;* three ocelli are present, and the peculiar genitalia foreshadow those of the Tabanidæ and Leptidæ, while as I have already pointed out those of the Asilidæ, Stratiomyidæ and Dolichopodidæ and Empidæ seem to have been derived from a form like that of *Tipula*.

I venture to express these ideas in the shape of a tentative scheme, as follows:


Muscidae
Conopidae
Syrphidae
Bibionidae
Stratiomyidae
Asilidae
Empidae
Dolichop
Phoridae
Lonchoptera
Tabanidae
Bombylidae
Leptidae
Scatopsinae
Tipulidae
Ptychoptera
Rhyphidae
Other families
Simulidae
W's 2nd Phylum
Panorpidae
Primitive Diptera
Metabola

If we are content to accept this view that the Rhyphidæ are close to the ancestral form of the Tabanidæ, we get another gleam of light on this obscure pedigree. If the idea is tested by the general morphology of the families it will be seen one section is characterized by a comparatively large head, flat thorax and broad, flat abdomen; this includes the Rhyphidæ, Simulidæ, Tabanidæ and the Cyclorrhapha. The other section has a small head, humped thorax, and a narrow, depressed abdomen; this includes the Tipulidæ, and the Brachycera, except the Tabanidæ, Leptidæ, Therevidæ and Bombylidæ. The persistence of these two well-marked forms is good evidence of my argument, and, though it weakens the status of Williston's fifth phylum it establishes even more strongly his first and second.

On the Classification of the Nemocera.

If the table of the mouthparts of the older families is examined it will be seen that in only one family, the Cecidomyidæ, is the pharyngeal pump absent, but my specimens of this family are so few in number that probably this observation has no value. I feel confident that, with sufficient material, it will be demonstrated in some genera, even though vestigial. But, I am sure that *Bibio*, *Dilophus* and *Chironomus* do not have it, though it is present in *Scatopse* and some *Ceratopogones.* Not only are the Bibionidæ singular in this respect, but the eye structure, the limbs and bristle structure are all highly specialized. The usual coloration of certain species is singular and as remarkable as anything among Diptera, while the simpified venation and modified mouthparts mark them off as the most specialized family among the Nemocera. The only archaic characters remaining are the ocelli, the four-jointed palpi, and the long, membranous labium of *Dilophus*, with the palpi inserted near its end. In comparing the Chironomidæ with the Bibionidæ it will be seen that the mouth is less specialized, since the stipites and cardines are obvious and the mandibles are not fused, though embedded in the ventral side, as in *Bibio* and *Tipula;* the eyes are quite archaic in type, as is also the bristle and hair structure. Though the Culicinæ are highly specialized, the Corethrinæ are obviously older, and they possibly represent the continuance of an ancestral form of the Chironomidæ, as the venation is archaic and the mouth retains the pharyngeal pump.

These points show that the present arrangement of the families of the Nemocera, though convenient, is not founded on a true principle. An arrangement which collocates those families possessing the most archaic characters would seem to be more proper. I would arrange Williston's groups 1 and 2 at the head of the Nemocera as follows:

1. Rhyphidæ.
2. Simulidæ.
3. Cecidomyidæ.
4. Mycetophilidæ.
5. Orphnephilidæ.
6. Psychodidæ.
7. Tipulidæ.
8. Dixidæ.
9. Culicidæ.
10. Blepharoceridæ.
11. Chironomidæ.
12. Bibionidæ.

I would place the Rhyphidæ first, since the venation is archaic, complete ocelli are present, the size is small and the pubescence and leg bristles are primitive. Add to these the structure of the mouthparts which appear to have their prototype in the Myriapoda, and it appears to me that the evidence is overwhelming that the family represents the most archaic type of Diptera; but I shall have more to say on this subject later.

The Simulidæ follow, as they also possess the peculiar structure of the palpi and have a nearly complete mouth-armature and are small in size. The Cecidomyidæ come next on account of the many-jointed antennæ and the archaic type of eye structure, but I admit that their place in the scheme is tentative. The Mycetophilidæ are difficult to separate from the Cecidomyidæ, but their eye structure, mouth, tibial bristles and the variable condition of the venation all justify their position here. The Orphnephilidæ are usually placed next to the Psychodidæ, which is a very old type retaining many archaic characters in eyes, mouth and wings.

The Tipulidæ follow as preserving the oldest type of venation, of leg pubescence and bristle-structure, together with the old types of eye structure, mouth and genitalia. The Dixidæ follow as the first family of Williston's second phylum. It is difficult to say whether the Culicidæ or the Chironomidæ should have the precedence. The former are entitled to it on the venation (an obviously old form, possibly ancestral) and on the mouth, though I have a *Ceratopogon* in my cabinet with pharyngeal pump and broad-bladed maxillæ and mandibles, which is more primitive in type than the armature of *Culex*. But this is exceptional, the majority of the *Ceratopogones* being without mandibles and having the laciniæ of the maxillæ of a simpler type. The archaic type of eye structure is matched by *Corethra* and the absence of a pharyngeal pump in so many genera, whereas it is always present in the Culicidæ, decides in their favor.

The Blepharoceridæ follow, and the Chironomidæ after. I have already given my reasons for placing the Bibionidæ last.

Williston, in the true spirit of a paleontologist, has speculated on the primitive dipteron, and has given in words a reconstruction of a hypothetical form (p. 331, 2), as follows: "The primitive dipteron must have had eight fully developed longitudinal veins (including the auxiliary vein) with the second, third, fourth and fifth furcate, and a complete discal cell. The head was rather small, with the compound eyes separated equally by the front in both sexes. The ocelli were functional, and the maxillary palpi had four freely articulated joints; the labial palpi had probably already disappeared, though Weschè thinks differently. There were at least thirty-nine antennal joints in the male. The prothorax, mesothorax and metathorax were imperfectly fused, and the metanotum was visible from above. The abdomen had nine functional segments; the body was without differentiated bristles; and the tarsi had membranous pulvilli and empodia. The primitive flies were of moderate or small size, and probably crepuscular in habit, or at least denizens of shady forests."

Williston goes on to say that of modern Diptera the Tipulidæ approach most closely this hypothetical ancestor, principally in the venation, and remarks that they have become specialized by the almost complete loss of the ocelli, increase in size, and the loss of the pulvilli. He places the Rhyphidæ next in rank to the Tipulidæ. It seems to me, however, by his own diagnosis, that the Rhyphidæ are more primitive than the Tipulidæ. They are small, and have functional ocelli and pulvilli. The mouthparts are much less specialized (the mentum being developed) and they retain some remarkable archaic characters. I would protest against the time-honored custom of subjecting all other characters of Diptera to the venation.[1]

This reconstruction of the primitive dipteron by Williston has given me much pleasure and much food for thought, though I am not in agreement with him in his views of the labial palpi. I think that the primitive dipteron had, like all other contemporary insects, four palpi, and that they persisted in this condition until after the chief phyla had arisen, since a large majority of the Empidæ have what I believe to be undoubted labial palpi. I have no doubt that the structure of the labium in *Chrysops*, which I figured in 1904 in the cited paper on mouthparts, shows aborted labial palpi, the palpigers. Savigny, in the dawn of orismology, pointed out remains on the labium of *Tabanus italicus*, and I can show a number of preparations in the same family with tufts of hair in similar situations to the palpigers of *Chrysops*.

LOSS OF ANTENNAL JOINTS.

Williston discusses this subject on pages 328, 329 of the cited paper; some observations by myself may throw additional light upon it. I have in my cabinet a preparation of *Scatopse* of very small size, probably *S. minutissima* Verrall., in which the antennæ are unsymmetrical. The fourth and fifth joints are partially fused in the left antenna, the suture going only half through the segment; the right antenna has the full number nine of antennal joints, with the fourth and fifth separated (Figs. 1, 2); here we can clearly see that a middle joint has been lost. In preparations of *Dilophus* and *Bibio*, where, judging from the variations in number in different species, the antennæ are in an unstable condition I have several specimens where the distal joint consists of from three to six segments closely joined together, the last or true terminal joint in an atrophied condition (Figs. 3, 4). It would thus seem that, in the antenna of *Dilophus* at least, joints may be lost either by fusion of the middle ones or the disappearance of distal ones.

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[1] I will not quarrel with this conclusion, though I still think that holopticism outweighs in importance the archaic characters of ocelli and pulvilli and even of the mouth parts. It is quite evident, however, that the Rhyphidæ should no longer be placed at the extreme end of the Nemocera.—S. W. WILLISTON.

THE CHARACTER OF THE HAIRS AND BRISTLES ON THE LEGS OF DIPTERA AND OTHER INSECTS.

In the striking reconstruction of the primitive dipteron I have quoted, Williston has suggested that the body was without differentiated bristles. This character may well be extended to the limbs also. In 1902 I published some figures of the legs of diptera,[1] but these were mainly concerned with the strangest forms I could select; though the hairs and bristles were arranged in striking forms they were mostly subsidiary and depended on the altered shapes of the femora, tibiæ and tarsi. Later, in 1908,[2] I gave twelve figures of the microscopic appearance of preparations of legs taken from twelve different flies, three to illustrate a simple type, four the raptorial type, four the secondary sexual type, and one the parasitic type. The study of the limbs has led me to place considerable reliance on the hair and bristles as characters, and I find myself quite in agreement with Williston's idea that the simpler pubescence is the older form. My selection, a purely chance one, gave me as a result the legs of a tabanid, a leptid, and a stratiomyid as simple types. Going further back in an endeavor to realize what the primitive characters might be, I examined preparations of Myriopoda, *Blatta*, *Forficula* and *Panorpa*. These showed very wide differences in the bristles and hair with which they were more or less covered. Of the Myriopoda five species were examined, two Indian (Kashmir) and three British. A large *Scolopendra* is without pubescence, and with only two small bristles at the penultimate joint of the tarsi and two at the base of the claw. A species of *Scutigera* has an extraordinary number of tarsal joints (39) covered with short hairs, some of them short and stiff, with bristles at the larger joints, the parts that may represent the coxæ, femora and tibiæ. Two British species (*Cryptops*) have short, stiff hairs regularly disposed over the legs; but a larger and broader species with a greater number of legs has them almost bare.

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[1] "Modifications of the Legs of Some Dipterous Insects." *Journal Quekett Club.*

[2] "On the Microscope as an Aid in the Study of Biology in Insects." *Journ. Royal Microscopic Society*, August, 1908.

In *Blatta* a few short hairs are scattered over the limbs, but the femora and tibiæ are armed with many strong, sharp spines, which, in the genus *Phyllodromio*, are serrated with minute but regular barbs, undoubtedly specialized for raptorial purposes. In *Forficula* there are no bristles, only minute, soft scattered pubescence, which is much thicker on the inner side of the tarsi than elsewhere. In *Panorpa* a short, very even, uniformly long and regular pubescence is found studded with longer spines on the tibiæ and tarsi, and with tibial spurs of a curious and marked structure, each spur appearing as if it were made up of a number of fine hairs of various length, so that the edges appear almost plumose, certainly serrate.[1]

It may be of interest to record that *Peripatus novazealandiæ*, that remarkable survival, has neither pubescence nor bristles on its short forelegs or on any part of the skin, which, however, is studded with minute papillæ.

All these arthropods except *Peripatus* have one character in common, and that a very marked one. From the upper joints, or femora to the claw or claws, there is seen what under low magnification appears to be a thread-like tendon, but under high magnification a duct leading to the claw, either carrying poison to the claw or moistening the plate at the base of the empodium, and from that part the pulvilli. This duct might have been described from diptera instead of *Blatta*, *Forficula* or *Panorpa* so obvious are the homologies, but the arrangement of hair and bristles on the surface suggests no counterpart, except in *Panorpa*. A comparison of my preparations in the Nemocera with that insect shows that the pubescence of the legs approximated closely in *Gynoplistia bella*, particularly in the region of the tarsi; in *Ptychoptera albimana*, *P. lacustris*, *P. scutellaris*, and *Rhyphus fenestralis* the structure of the tibial bristles is practically identical. This is certainly remarkable, as Woodworth on the evidence of the venation[1] has suggested that this family is more closely related to the diptera than any other, branching off after the Neoptera had left the Metabola.

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[1] In my paper on the systematic affinities of the Phoridæ in the *Transactions of the Entomological Society*, I stated that this structure was only to be found in the Mycetophilidæ and Phoridæ. I should have stated that only in the former family were they found in a size comparable and requiring a magnification of 250 diameters for elucidation. These on *Panorpa* and the diptera mentioned later are much larger and can be seen with lower powers, except in the case of the Rhyphidæ, which is a recent observation. Of course the presence of this structure in other insects admittedly of ancient type only strengthens my former argument, but it also shows the danger of dogmatic formulas.

I think that we may assume that the primitive type of pubescence on the legs of diptera was somewhat similar to that which yet exists in *Rhyphus* and the Tipulidæ; and that when marked bristle structure or armature is found the insects are specialized. So we recognize, and this harmonizes well with other characters, that the Bibioninæ and the Culicinæ are the most specialized subfamilies among the Nemocera, such forms as *Dilophus*, or *Mucidus* and *Sabethes* making this clear. In the Mycetophilidæ, *Sciara* preserves the older type, while *Mycetophila* in the strong spines on the tibiæ and tarsi is more specialized, which idea is quite confirmed by the ocelli, three in *Sciara*, two in *Mycetophila*, and these remote from their usual position. The other families (I am not certain of the Blepharoceridæ) are all of the simpler types as is the genus *Scatopse* and the subfamily Corethrinæ.

Among the Brachycera, in the families with many genera, a number of variations between simplicity and complexity will be found, mostly as secondary sexual characters in the male, while the predaceous insects will be found modified in both sexes. The more striking examples of the latter will be found among the Empidæ, the Asilidæ appearing to confine their armature mostly to the tarsi. The Phoridæ have a peculiar and characteristic chætotaxy, but do not vary markedly; while the Leptidæ, Stratiomyidæ, Tabanidæ and Cyrtidæ are all of the simpler type, the Leptidæ most nearly like the Tipulidæ, retaining the peculiar bristle structure. The Platypezidæ and Pipunculidæ show various modifications of rows of long bristles or hairs as well as peculiarly modified bristles. In some Platypezidæ these bristles

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[1] "Wingveins of Insects." Univ. California Publications, Entomology, Vol. 1, p. 145, 1906.

(as in *P. consobrina*) are, like the modified hind tarsi, found in both sexes.

Among the Cyclorrhapha, as might be expected, we find the characters of the legs extremely developed. Among the Syrphidæ, though there are many such simple forms as *Chilosia*, we find progressive degrees leading to great complexity, as in *Platycheirus* and *Pyrophæna*. Such a form as *Sphærophoria scripta* is an intermediate one; the general type is simple, but the under side of the middle femora of the male is studded with short sharp hairs absent in the female. The four genera of the Conopidæ examined all show a greater specialization than *Sphærophoria*. *Gastrophilus equi* has a long shaggy pubescence far removed from the simple forms. Of the Muscidæ alone a chapter might be written on the variations of the pubescence and bristles of the legs; and some flies, like *Glossina*, have structures which appear to be characteristic. All these modifications are those of strong bristles, though softer hairs are often present. I can not call to mind instances, unless it be *Calobata* where the pubescence is uniformly like that of *Gynoplistia* and the Panorpidæ, soft and weak.

On Certain Glands in the Mouths of Some Myriopoda and Diptera.

In the limb-like maxillæ of *Scutigera* (a centipede with compound eyes) there are organs of striking structure. In addition to the poison glands, which may easily be mistaken for tendons or overlooked, there are transparent chitinous bulbs communicating with apertures in the claws by ducts of moderate length. These bulbs are studded with a number of short tubular processes which show clearly when the edges of the organs are focused. These are peculiar structures of characteristic appearance, and are very unlikely to be confounded with other organs. I naturally reached the conclusion that these were poison glands, as the bite of the centipede, in addition to the punctures of the claws, is known to be poisonous. With this idea I was surprised to find in the maxillary palpi of *Rhyphus fenestralis* ♀ a similar structure which I have figured in the cited paper on mouth-parts, as sense organs. These communicate with the air by fairly large openings in the walls of the palpi (Fig. 14). Unfortunately I have no preparation of the male of this species, but I find a similar structure in the male of *R. punctatus*, though smaller in size and with a shorter duct; as also in a Tasmanian species as well developed as in the former species. The differences between these two species in this respect are so marked that it is possible to separate them on this character alone (Fig. 13).

The uses of the organ are obscure, but they are probably similar in both the myriopod and the insects. The poison duct in the legs is quite similar to that part in the maxilla; it traverses many joints and opens underneath the claw between two bristles inserted at its base. Without a doubt it is the homologue of the duct which moistens the pulvilli in the flies. Again referring to the maxillary gland, my observations have not ended here, as the palpi of *Simulium reptans* ♀ and *S. ornatum* ♂ have similar structures, though they communicate with the air by a different opening. Moreover in the mouth of two British species of *Cryptops*, on the maxillæ, or more properly speaking the maxillipeds, and absolutely homologous in situation and structure, are similar glands to those found in the Indian *Scutigera*.[1]

That this structure should be found surviving in Diptera is exceedingly remarkable, but not more so than the fact that the duct which leads the poison to the many claws of *Scoliopendra* should be found in a precisely similar condition in nearly all insects. I have found it throughout Diptera, in *Blatta*, *Forficula*, *Panorpa*, Lepidoptera, Hemiptera, in fact in all insects where there are membranes on the claws that need irrigation.

I have studied the sense organs of insects for many years, and by comparing the large number of preparations, using modern optical methods and objectives I have become familiar with their appearance in the antennæ, palpi and mouths of Diptera, and many other insects. Unless I am greatly mistaken, and mistakes are easily made in such minute structures, I can say with confidence that there are found in the palpi of *Rhyphus* and *Simulium* homologous organs of peculiar structure; and judging from a comparison with *Scutigera*, this character is one of the most ancient hitherto observed in Diptera, a character which existed before there were winged insects, and consequently before venation, a character which is quite in agreement with the idea that the Rhyphidæ are among the most archaic types, the least specialized of all flies.

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[1] I find the structure in a modified form in a small *Lithobius*, found in a garden in London.

Summary of New Observations.

1. Ankylosis of middle joints of antennæ of *Scatopse*.

2. Fused distal joints in the antennæ of *Dilophus*.

3. Observations on the legs of myriapods and insects.

4. The tibial bristles of *Panorpa*, the Rhyphidæ, Mycetophilidæ, Tipulidæ, Leptidæ and Phoridæ are all of the same peculiar structure, and, excluding the Phoridæ, the general pubescence of the legs is approximately similar.

5. A peculiar structure exists in the mouths of some Myriapoda, and similar structures in the palpi of the Rhyphidæ and Simulidæ.

6. The pharyngeal pump has been found in the heads of the Rhyphidæ, Psychodidæ, a *Ceratopogon*, *Scatopse* and the Simulidæ; and it has been dissected out in a vestigial condition from the heads of *Hæmatopota pluvialis* and *Tabanus africanus* ♀.

7. A *Ceratopogon* with mandibles has been found.

8. An observation on the number of receptacula of *Lonchoptera flavicauda* is recorded.

9. A suggestion is offered that the peculiar genitalia of the Rhyphidæ have some affinity with those of the Tabanidæ and Leptidæ.

10. The mentum is fully developed in the Rhyphidæ, another important link connecting the Nemocera with the Brachycera.

Explanation of Plate.

Fig. 1. Di gram of left antenna of *Scatopse minutissima*, to show the partial ankylosis between fourth and fifth joints.

Fig. 2. Diagram of the right antenna of same insect, showing normal structure.

Fig. 3. Diagram of antenna of *Dilophus febrilis*, showing condition of distal joint.

Fig. 4. Diagram of antenna of *Dilophus albipennis* to show condition of distal joint.

Fig. 5. Gland (?) from the maxilla of *Scutigera*, highly magnified. Its situation is shown in Fig. 12*a*.

Fig. 6. Second tarsal joint of hindleg of *Gynoplistia bella* (Tipulidæ) to show pubescence under magnification of 60 diameters.

Fig. 7. Second tarsal joint of *Leptis scolopacea* under like magnification.

Fig. 8. Tibial bristle of hindleg of *Panorpa communis*. 60 diameters.

Fig. 9. Tibial bristle of hindleg of *L. scolopacea*.

Fig. 10. Tibial bristle of hindleg of *G. bella*.

Fig. 11. Second tarsal joint of hindleg of *Panorpa communis*, as seen with magnification of 60 diameters.

Fig. 12. Diagram of a segment from the mouth of *Scutigera* to show the maxillæ and situation of the poison (*b*) and other glands (*a*).

Fig. 13. Second joint of maxillary palpus of *Rhyphus punctatus*. Diagram in optical section to show gland (*a*) with duct opening in the anterior portion of the joint, and the sense organ which is probably olfactory (*b*), as seen with magnification of 300 diameters.

Fig. 14. Trophi of *Rhyphus fenestralis* showing the ventral side. The right palpus shows the sensory structure on its surface, while the left is drawn in optical section to show shape, situation and structure of the gland contained in interior, The mentum shows a distinct median structure, and below it are the submentum. and a portion of the pharyngeal pump. *m*, mentum; *sm*, submentum; *pp*, pharyngeal pump; *a*, gland; *l*, lacinia of maxilla. Drawn from several specimens mounted with and without pressure, showing structure under magnification of 300 diameters.

Fig. 15. Hypopharynx of *R. fenestralis* showing submentum, an unusual condition.

Fig. 16. Labrum of *R. fenestralis*.

Fig. 17. Second joint of palpus of *R. brevis* Walker, differing from *R. punctatus* in the size and attachment of the gland (*a*) and the character of the sense organ (*b*) and from *R. fenestralis* in the attachment of the gland to the wall of the segment, and in the character of the sense organ. Same magnification as Fig. 13.

Fig. 18. Tibial bristle of hindleg of *R. fenestralis*. Magnification of 300 diameters.

Fig. 19. Tibial bristle of hindleg of *R. punctatus*. Same magnification.

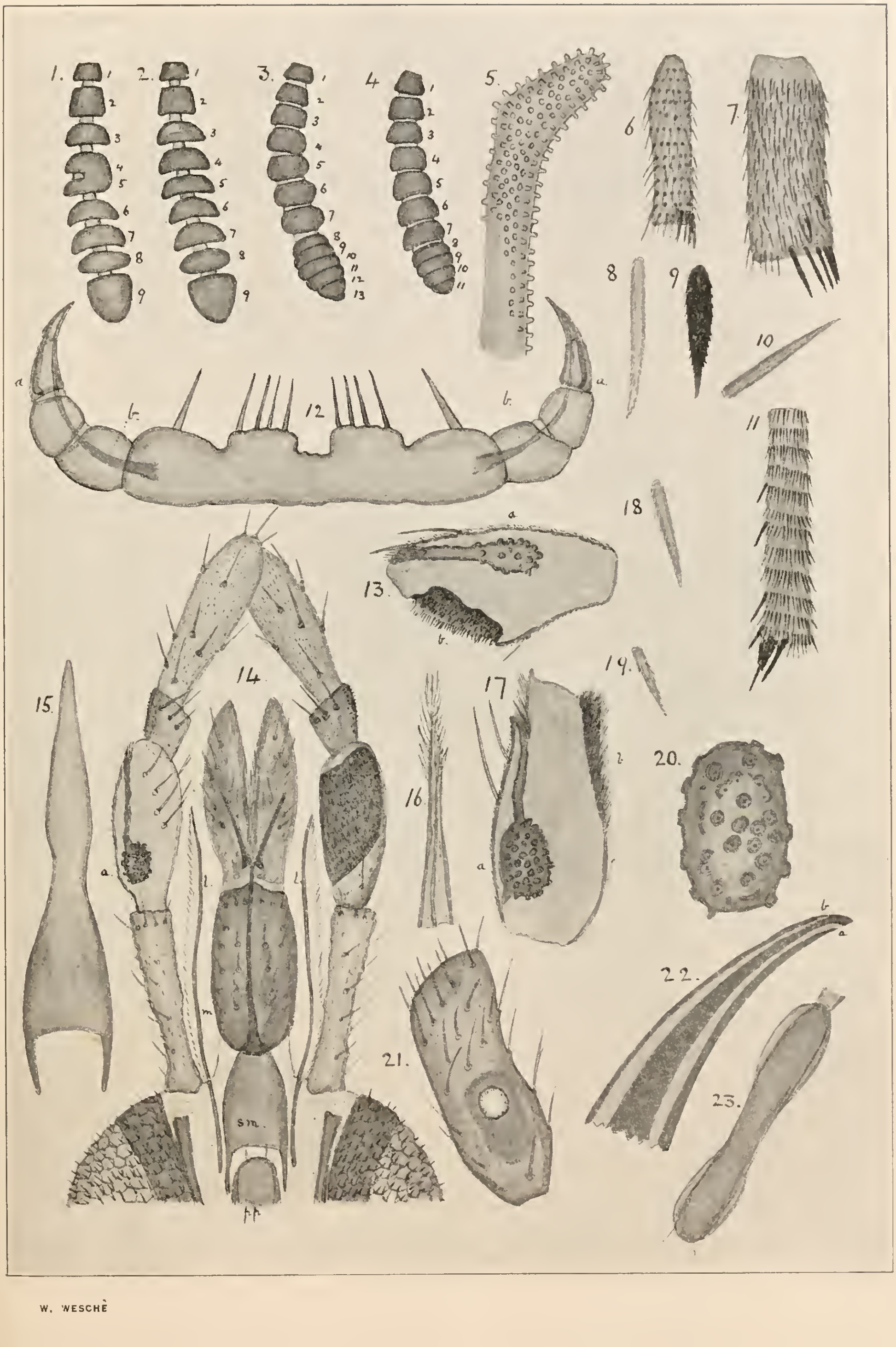
Fig. 20. Gland from interior of second joint of palpus of *Simulium reptans*, to show structure on side opposite the opening.

Fig. 21. Second joint of palpus of *S. reptans*, highly magnified.

Fig. 22. Point of maxilla of *Scutigera*, seen in optical section and highly magnified, showing openings of the poison duct (*a*) and of other gland (*b*).

N.B. The size of the figures has no relative significance.

PLATE I.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W. WESCHÈ

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BIOLOGICAL BULLETIN

THE UTERINE SPINDLE OF THE POLYCLAD PLANOCERA INQUILINA.

J. T. PATTERSON AND H. L. WIEMAN.

I. INTRODUCTION.

In the uterine eggs of several species of turbellarians there appears a large conspicuous spindle to which the name "uterine spindle" is applied. It is also known under the terms "disappearing spindle" and "aborting spindle." The appearance of this spindle in the uterine eggs of these worms would not in itself be so striking were it not for the remarkable statements that have been made concerning its subsequent history. It is the general opinion of those who have observed the spindle that upon reaching the metaphase it breaks down to form a resting nucleus, which in turn gives rise to the first maturation spindle.

The uterine spindle was first described in 1881 by Selenka, in connection with his work on *Thysanozoön Diesingii.* He states that it first makes its appearance in the eggs after they have become full grown and have passed into the uterus, and is preceded by changes that are typical of the first maturation mitosis. Thus the chromatin of the germinal vesicle forms a spireme, the achromatic spindle with centrosomes appears, and the chromosomes pass into the equatorial-plate position. At this point the division process is arrested, and the "polar-suns" draw closer together, become indistinct, and the chromosomes fuse. The whole nucleus finally returns to a resting condition. Later, the egg gives off two polar bodies, is fertilized and proceeds to develop in the normal manner. Selenka concludes that this incomplete karyokinesis occurs in order to effect a massing of the yolk granules about the astral centers.

Lang ('84) seems to have been the next observer to have noted the uterine spindle. He describes it as appearing in several species of polyclads; and while he does not accept Selenka's theory as to its function, nevertheless he regards it as a part of the normal process of the nucleus.

In 1894 Wheeler observed the uterine spindle in the eggs of *Planocera inquilina*, a polyclad inhabiting the branchial chamber of *Sycotypus canaliculatus*. Wheeler was concerned with a description of this new polyclad, and did not attempt to work out the details of the karyokinetic process. Since *Planocera* is the form with which this paper is concerned, we may quote Wheeler's entire but brief statement on the aborting spindle. His statement is as follows: "As soon as the mature ova pass into the uteri a curious phenomenon, first seen by Selenka in the uterine eggs of *Thysanozoön Diesingii*, may be observed. The wall of the germinal vesicle fades away and a spindle is formed with distinct polar suns containing centrosomes. The small chromosomes, nine or ten in number, form an equatorial plate and appear to undergo fission, but of this I am not certain. Then the polar asters grow faint and vanish and the nucleus returns to the resting stage during or just before oviposition. Before the nucleus has returned to the resting stage the spermatozoön enters the egg. I have several times seen the deeply staining and somewhat twisted head of the spermatozoön lying in the cytoplasm near the arrested spindle. Further than this I have not traced the phenomena of impregnation, as my attention was first attracted to them while studying hardened material when I was far from the sea-shore. Why a spindle should be formed in the mature ovum and no division result, but only a return of the nucleus to its resting stage, is not easily understood. The spindle lies in the center of the egg and has nothing to do with the formation of the polar bodies; for these do not appear till some time after the eggs are laid, as I have several times had occasion to observe." Wheeler concludes his account of the spindle by objecting to Selenka's view concerning its supposed function.

Gardiner, '95 and '98, studied the uterine spindle in the acœlan *Polychœrus caudatus;* but in this form it is clearly the first cleavage spindle, as the polar bodies are thrown off before it makes its appearance. However, according to the description of Gardiner the behavior of this spindle is similar to that of the uterine spindle of the polyclad egg. Thus he states that if the animal be kept too long under adverse conditions, the polar suns of the spindle grow dimmer, draw closer together, and the nucleus appears to return completely to a resting stage. The egg remains in this condition until after it is laid, when the spindle again appears, this time to initiate the process of cleavage.

Through certain experiments, Gardiner, '98, demonstrated that the retrograde growth of the amphiaster of the uterine egg was due to placing the animals under adverse conditions, which in turn caused a delay in the laying of the egg, and under such circumstances development begins, as indicated by the appearance of this spindle. Furthermore, he clearly showed that the uterine spindle of *Polychœrus* follows maturation, and is therefore, as stated above, the first cleavage spindle. Through the results of these experiments, Gardiner is led to suggest that the so-called uterine spindle of the polyclads is probably the first segmentation spindle. His exact position on this point may be gleaned from the following quotation: "I would suggest, therefore, that individuals of the polyclads, in which such structures are found, have before death been placed under some abnormal conditions; that the ovum has been fertilized and the polar bodies formed; that the first segmentation spindle has been formed; and that the environment was such that oviposition could not take place; consequently, that a retrograde development of this spindle has taken place exactly as in *Polychœrus*."

That the ground for this suggestion is not well taken, we shall later try to demonstrate, at least in the case of *Planocera*. The suggestion of Gardiner, however, should make us cautious about regarding as aborting spindles those that are visible in the uterine eggs of several worms figured by von Graff ('82 and '08) and which a number of investigators have cited as examples of the disappearing spindle.

In 1907, Surface, who studied the early development of *Planocera*, also called attention to the uterine spindle in this animal. He did not attempt to work out the history of the spindle, but gives merely an outline figure of a freshly laid egg, in which is seen what he takes to be a "germinal vesicle" produced by the retrograde development of the uterine spindle. Surface states that if the spindle is an abnormal display, as claimed by Gardiner, it at any rate does not interfere with the normal process of development.

The last account of the uterine spindle is that on the rhabdocœle *Graffilla Gemellipara*, which was studied by one of the present writers (Patterson, '12). In this animal a large conspicuous spindle was occasionally met with in eggs that had not yet undergone maturation. The spindle in *Graffilla* differs from that so far observed in any other worm, for in practically every case it exhibited some peculiar condition, such as the abnormal position of the chromosomes on the spindle fibers, or even their complete absence from the spindle. It was pointed out that on account of the viviparous mode of reproduction, *Graffilla* was not a favorable form in which to study the history of the aborting spindle. It is impossible to secure a complete series which would show conclusively the exact progress of its development.

To sum up: The term uterine spindle has been applied to a variety of karyokinetic phenomena which occur during the first steps of development. Some of these cases are undoubtedly due to abnormal development; others are not, especially those of the polyclads. Here the general verdict seems to be that the uterine spindle appears before maturation, that it does not go beyond the equatorial-plate stage, and that it subsequently retrogrades to produce a sort of resting nucleus, which in turn develops the first maturation spindle.

It is evident from the above brief review of the literature that a great deal of obscurity exists regarding the uterine spindle. In view of the fact that no one has given a consistant account of its development, and in view of the further fact that no rational function has been assigned to it, we have considered it worth while to make a detailed study of this spindle. To do this we have selected *Planocera*, not only because of the ease with which this animal can be secured, but also for the reason that its egg is supposed to contain the most typical example of this apparent anomaly of cytology.

Material and Methods.—Two or three is the most common number of worms found in a single whelk, although we have secured as many as eight from one specimen. After removing the shell, the branchial chamber of *Sycotypus* is slit open and the worms removed to dishes of fresh sea water. This operation was done very shortly after the animals were brought into the laboratory.

When the eggs are fully matured the polyclad lays within several hours after being transferred; otherwise twelve or even twenty-four hours may elapse before eggs are deposited. Usually the eggs are laid in a helicoid spiral on the bottom or sides of the dish, as Wheeler ('94) and Surface ('07) have observed; but quite often oviposition occurs beneath the surface film of the water, in which case the egg string takes the form of a slightly curved ribbon. In the latter instance the worm lies with its ventral surface upward, in which position one can readily study the entire process under the binocular. The eggs are forced out by rhythmic contractions of the egg ducts, and at the same time embedded in a perfectly transparent gelatinous substance of a very sticky consistency. The average time for the act of oviposition is about 15 minutes. Each egg is provided with a delicate capsule, probably secreted by the shell gland surrounding the egg duct. Occasionally a single capsule incloses two eggs. A string may contain as many as 2,000 eggs. Adult worms were obtained showing eggs in every stage of development from the beginning of the growth period up to the time of laying. Eggs were killed just at time of oviposition and at fifteen minute intervals for several hours afterward. In this way a complete series of stages covering the entire period of growth and maturation was obtained.

Adults and eggs were killed in the bi-chloride-acetic-formalin mixture described by Bartelmez ('12).

SOLUTION 1.

Saturated solution of .7 per cent. NaCl	94 c.c.
Glacial acetic acid	6 c.c.

SOLUTION 2.

Neutral formalin (commercial formaldehyde neutralized with $MgCO_3$)	10 c.c.

The two solutions were kept separate until the time of using.

The worms were killed in the mixture heated to 50° C. and left for one and one half hours. The eggs were treated with the cold solution for about an hour. Excellent fixation was obtained, and the material proved very favorable for cytological study.

When the eggs were laid on the glass, they were allowed to remain until 80 per cent. alcohol was reached, and then carefully taken off with a sharp scalpel. An entire string may be removed in this way without losing or injuring a single egg.

The adults and eggs were embedded and cut in hard paraffin (60° C.) to which sufficient quantity of a rubber-asphaltum-paraffin mixture was added to produce a light amber shade. Sections of 5, 7 and 10 micra thickness were cut without difficulty.

Heidenhain's iron-alum-hæmatoxylin with orange G gave very good results, but the method was in all cases checked by staining parts of series with safranin and Lichtgrün.

II. Formation of the Spindle.

The nucleus of the egg has a very characteristic appearance throughout the growth period. A coarse reticulum containing varying amounts of chromatic material at its nodal points, depending upon the degree of maturity of the ovum, and a large spherical basic-staining nucleolus are always present (Fig. 1).

Fig. 2 shows an early stage in the prophase of the uterine spindle, in which the chromosomes are forming at various points in the reticulum. Our preparations clearly show that the entrance of the spermatozoön into the egg is the stimulus which initiates the process, and that uterine eggs in which a careful examination fails to reveal the presence of a sperm invariably have the nucleus in the resting condition. Owing to the hypodermic method of insemination (Wheeler, '94) all the tissues of the body at this time are filled with spermatozoa which finally work their way to the uterus where they penetrate the ova. In the impregnated ovum the head of the spermatozoön stands out very distinctly as a deeply stained sickle-shaped rod, sharply pointed at one end, so that its presence can be easily recognized.

In Fig. 3 the chromosomes are fully formed in a group about the nucleolus which at this time stains very faintly. The nuclear membrane, though still intact, is somewhat wrinkled in outline suggesting that certain substances are passing out of the nucleus into the cytoplasm.

Fig. 4 shows the chromosomes scattered irregularly on the spindle, and it will be noted that the chromosomes are of a bivalent type. The centrosome is seen at one pole as a deeply staining sphere, in the astral area of which the spermatozoön lies. The section does not pass through the centrosome of the other pole. The chromosomes quickly move to the equatorial region and the spindle remains in this condition until the egg is laid. Fig. 5 is a polar view of such a spindle and shows the number of chromosomes to be ten, a number confirmed by many counts. Fig. 6 represents the characteristic appearance of a section of a uterine egg showing the entire spindle with the chromosomes in the equatorial plate. The spermatozoön is present in the next section of this egg.

Such in brief is the history of the formation of the uterine spindle. It is an enormous structure occupying the entire central region of the egg; its astral radiations stretching out from either pole almost to the egg membrane. The centrosomes appear as deeply staining spheres, in iron-hæmatoxylin preparations, but in safranin-Lichtgrün each sphere is resolved into a number of hollow vesicles staining with the acid dye.

At this time the spermatozoon may be seen in almost any part of the egg, between the spindle and the periphery. It may lie in the astral area or very close alongside of the spindle and near the chromosomes. The spermatozoön shows no change in structure from the sickle-shaped form in which it first appears in the impregnated ovum.

This is the type of spindle formed in the vast majority of uterine eggs, and in animals secured under the best conditions, only this type is found. However, in some adult worms, in addition to this so-called normal spindle, there occur other spindles which have the same general structural features, but show anomalies of various sorts. Thus the axis of the spindle may be bent (Fig. 7) or even broken at the equator and the chromosomes may be scattered irregularly on or near the spindles. Tri- and tetra-polar spindles of a variety of forms are also found, two of which are shown in Figs. 8 and 9. None of these spindles so far as we have observed completes the division cycle; at least not while the ovum is still in the uterus.

We believe that these abnormal spindles are the result of unfavorable conditions arising principally from not removing the worms from the whelk soon enough after the latter are taken from the sea, or perhaps from some other pathogenic cause, for they do not have any part in the normal development of the egg. Whether or not such eggs develop after being laid is a question we have not entered into. It may be that the presence of a few such spindles among normal ones in the uterus has led other observers to believe that they were stages in the supposed disintegration of the normal uterine spindle. In fact, we were inclined toward such an interpretation until, largely as a result of exercising greater care in handling the living material, we obtained worm after worm in which the uterus does not show a single abnormal mitotic figure.

III. The Laid Egg.

The condition of the laid egg of *Planocera* has been described both by Wheeler ('94) and by Surface ('07). According to Wheeler, the nucleus returns to a resting condition during or just before the egg is laid, and Surface states that it contains a large germinal vesicle which is situated slightly to one side of the center. We are unable to confirm these observations. In the first place, we find a considerable variation in the condition of the freshly laid eggs. Usually such eggs show that the so-called uterine spindle has undergone, or is in the process of undergoing, contraction, just prior to its migration to the surface to give rise to the first polar body.

The varying conditions of which we have just spoken consist almost altogether in the state of contraction or shortening shown by the spindle at the time the egg is laid. The most extreme cases are those in which the shortening is completed and the spindle has already migrated to the surface of the egg. Indeed, we have one lot of eggs (laid July 25, 1912) which were killed immediately after they were laid, and in which one occasionally finds eggs having the first polar body well started or completely formed. These variations in the condition of the spindle are easily explained on the basis of the assumption that there is considerable variation in the time of oviposition on the part of the different individuals; and this in turn is undoubtedly influenced by the conditions under which the animal is kept just prior to the laying of the eggs.

We have been somewhat at a loss to account for the observations of Wheeler and Surface, but believe that they may be explained in any one of several different ways. If a lot of freshly laid eggs, in which the contraction of the spindle has progressed to an advanced stage, be examined under the low or medium powers of the microscope, many of the eggs will appear to possess germinal vesicles. However, it can be shown conclusively that under such conditions the small contracted spindle is practically invisible, and that what one really observes in these living eggs is the relatively clear area of protoplasm in which the small spindle lies. This can be shown beautifully by staining the fresh eggs with neutral red, and examining them under the 4 mm. objective. Under such conditions, the contracted spindle stands out with great brilliancy, and one can easily follow the course of its migration to the surface of the egg and observe the formation of the first polar body.

That eggs possessing germinal vesicles may be laid we do not deny, for occasionally they are; but we can affirm that such vesicles are never formed in an egg after it has produced the uterine spindle. They are merely the non-transformed germinal vesicles of ovarian eggs, and their presence at this stage is to be explained by the fact that eggs possessing them either have recently been penetrated by the spermatozoön, or have not been inseminated at all. There is no room for doubt on this point. These germinal vesicles are in every particular similar to those of the ovarian egg—so much so that we have deemed it unnecessary to draw one for illustration, but refer the reader to Fig. 1. Furthermore, we have found at least two freshly laid eggs which contained germinal vesicles undergoing transformation to form the first maturation spindle. Each of these eggs showed a condition that indicated recent insemination, for the spermatozoön was lying close to the egg membrane and had not yet undergone the transformation necessary to produce the vesicular condition, so characteristic of the sperms in the ordinary eggs, which already possess the completed spindle. It should also be noted that we have occasionally observed non-fertilized uterine eggs which contained the unmodified germinal vesicle.

What we have just stated is further confirmed by observations on eggs that were fixed soon after they were laid. In certain phases of the contraction stage the spindle fails almost entirely to take up the hæmatoxylin stain, so that in studying such material one gains the impression that in at least some of the eggs the spindle has retrograded, or has even completely disappeared. However, if sections from the same series are stained with safranin and Lichtgrün the spindle stands out clearly and distinctly and is found in practically every egg.

This, together with the further fact that abnormal spindles are sometimes found, might easily lead the observer to conclude that a degeneration of the uterine spindle takes place. However, a careful study of a complete series of stages will convince anyone that such is not the case. We have been able, by the means of such a series, to follow the entire history of the uterine spindle, from the time of its first appearance up to the formation of the first polar body. The early phases of the spindle have already been sufficiently dealt with, and the rest of the history, from the contraction of the spindle to the formation of the polar bodies, follows.

The contraction of the spindle is a characteristic phase of the process of maturation, and occurs at about the time of oviposition or shortly thereafter. The spindle shortens to less than half its original length (cf. Figs. 6 and 10). During the shortening the astral centers of the spindle draw closer together and gradually stain less and less deeply. This is probably what Selenka and others refer to when they speak of the polar suns drawing together and growing faint. The end result of the shortening is the production of a short, relatively thick spindle. The shortening usually begins when the spindle starts to move to the surface, but in some eggs the process is completed while the spindle holds an approximately central position (Fig. 10).

This process of contraction is by no means unique for *Planocera*, but is characteristic of the first maturation spindle of several other forms, both among vertebrates and invertebrates. Conklin ('02 and '12) has described this same phenomenon in *Crepidula*, and in *C. plana* he states ('12) that the first maturation spindle undergoes a reduction from about 42 μ to 24 μ in length. He ('02) cites the following references in which the shortening process is known to occur: *Ascaris* (Boveri, '87), *Branchipus* (Bauer, '92), *Ophryotrocha* (Korschelt, '95), *Myzostomum* (Wheeler, '95), *Cerebratulus* (Coe, '99), *Polychœrus* (Gardiner, '98), *Axolotl* and *Triton* (Carnoy and Lebrun, '99, as seen in their figures 110 and 112). To this list we can now add *Planocera*, and also *Graffilla* (Patterson, '12).

Conklin's suggestion concerning the cause of this phenomenon is of interest here. He believes that it is due primarily to the peripheral movement of the spindle, and that its chief result is the formation of a much smaller polar body than would be produced if the spindle retained its original length.

During the progress of the contraction, the spindle moves toward the periphery of the egg, and upon reaching the surface its distal end comes in contact with the egg membrane, which apparently moves down to meet it. In the meantime the chromosomes undergo division, and the two groups have reached a late anaphase (Fig. 12). A protrusion on the surface of the egg then appears and into this elevation the distal end of the spindle is pushed (Fig. 13). Subsequently a typical polar body is cut off. We have found one unusually clear case of the first polar body, in which the ten chromosomes are easily seen (Fig. 14). Within another hour the second polar body is thrown off in the usual manner, and fertilization and cleavage then follow. In other words, the whole process of maturation and fertilization in this animal is quite typical of that of many other forms.

In conclusion we may state briefly the results of our study. We find that the so-called "uterine" or "aborting spindle" of *Planocera* is initiated by the process of insemination; that it is nothing more nor less than the first phases of a rather typical maturation spindle, and that consequently it undergoes a shortening while moving to the surface of the egg to give rise to the first polar body. It may be going too far to suggest that probably the uterine spindles which have been described in several other forms are of this same nature; nevertheless, we are inclined to believe that a careful study of the spindle in such organisms will show it to be only the first maturation spindle.

Woods Hole, Mass.,
August 3, 1912.

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Description of Plates.

Plate I.

The figures are camera drawings made at table level. Figs. 1–5 inclusive were made with 1.5 mm. Zeiss apochromatic objective and No. 12 compensating eye-piece; the remaining figures were made with the same objective and No. 6 eye-piece.

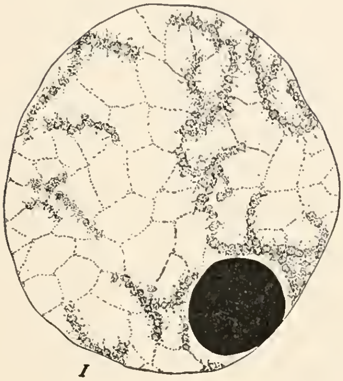
Fig. 1. Resting nucleus with large nucleolus characteristic of growth period.

Fig. 2. Early stage in prophase of uterine spindle. *C*, chromosomes.

Fig. 3. Prophase showing chromosomes fully formed about the fading nucleolus.

Fig. 4. Spindle showing typical tetrad chromosomes. *S*, spermatozoön.

Fig. 5. Polar view of equatorial plate of uterine spindle showing ten chromosomes.



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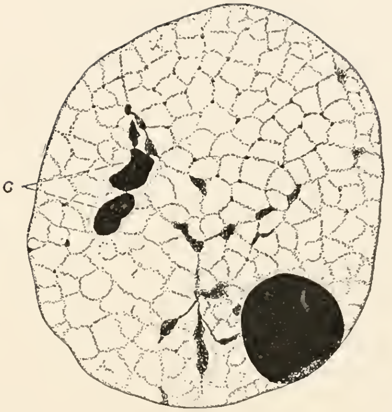

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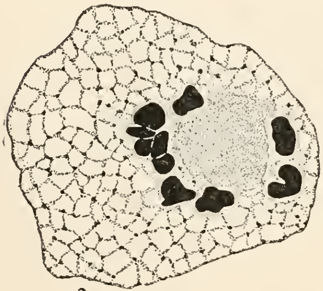

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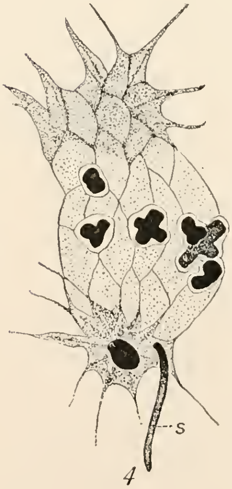

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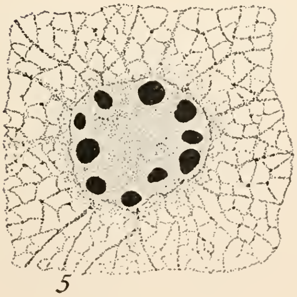

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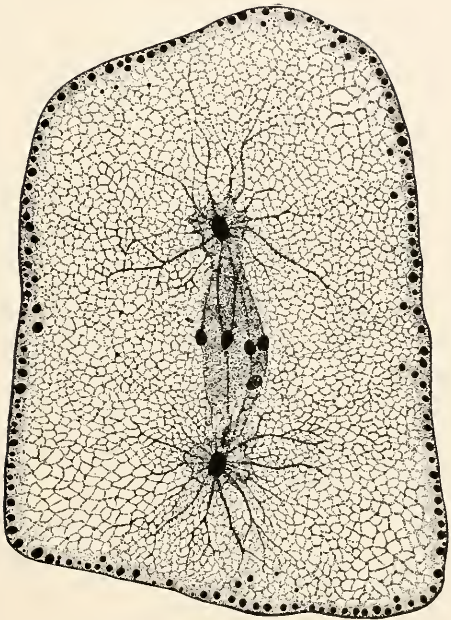
PLATE II.

FIG. 6. Section of entire uterine egg showing spindle.

FIGS. 7, 8 and 9. Abnormal spindles. *S*, spermatozoön.



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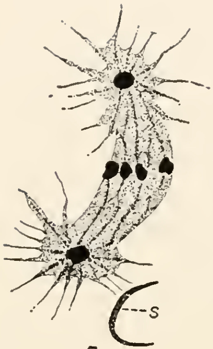

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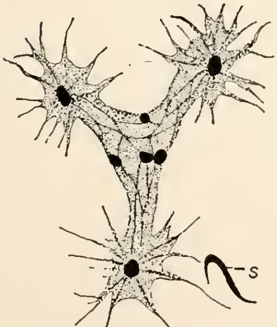

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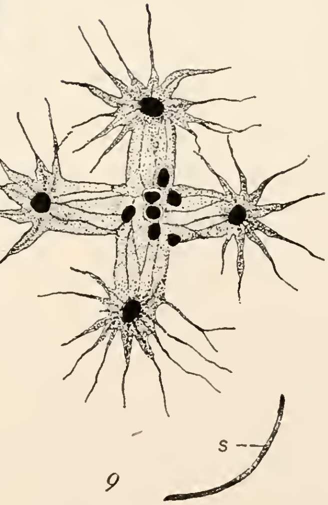

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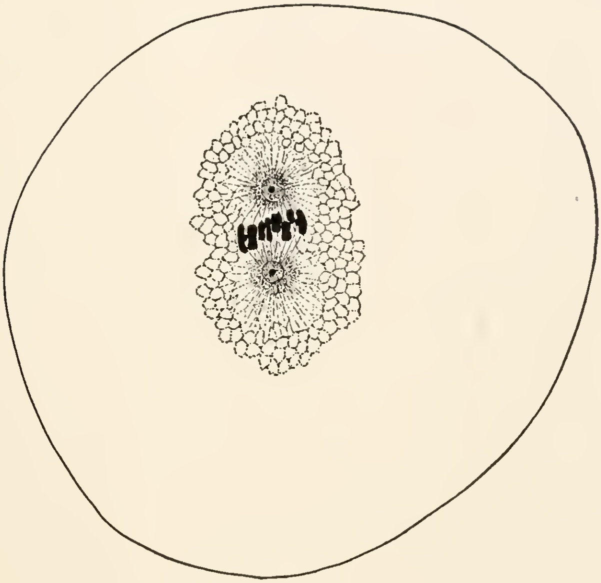
PLATE III.

FIG. 10. Laid egg showing spindle in contracted condition.

FIG. 11. Stages in migration of spindle to periphery of egg.



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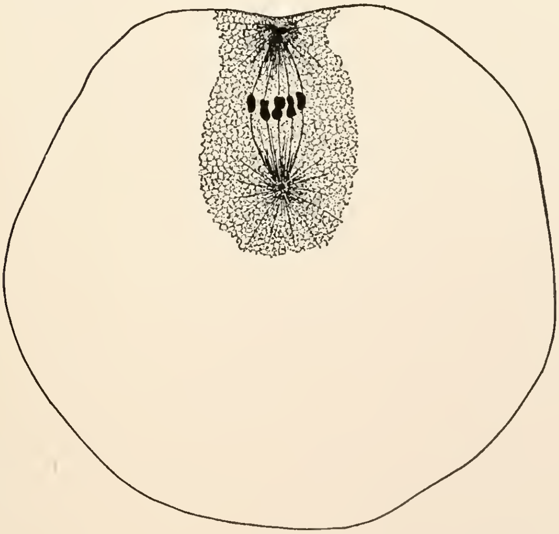


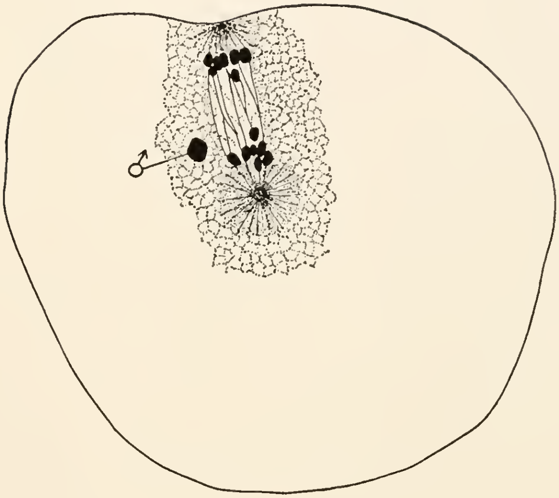
11

J. T. PATTERSON AND H. L. WIEMAN.

PLATE IV.

FIG. 12. Stages in migration of spindle to periphery of egg.

FIG. 13. Extrusion of first polar body.



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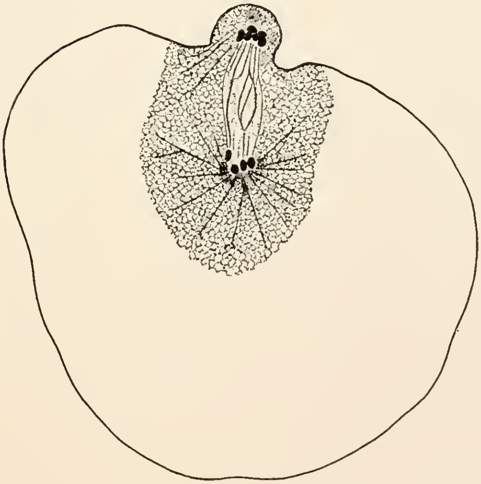

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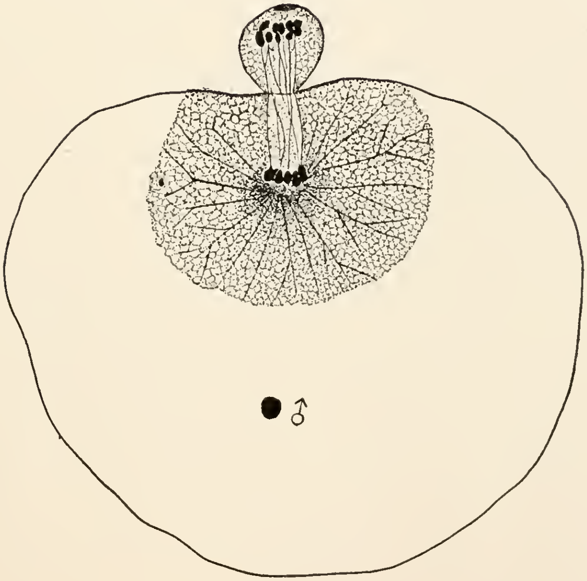
J. T. PATTERSON AND H. L. WIEMAN.

PLATE V.

FIG. 14. Extrusion of first polar body.



14

J. T. PATTERSON AND H. L. WIEMAN.

RESULTS OF HYBRIDIZING RING-DOVES, INCLUDING SEX-LINKED INHERITANCE.

BY R. M. STRONG.

I. Introduction.

The work which is described in this paper was undertaken at the suggestion of the late Professor Whitman. It was begun in a small way during the spring of 1904 and it was continued during the years from 1905 to 1909 with the hope that opportunities for more extensive breeding experiments might appear later. During the year 1910 it was decided to discontinue the work because there was still no prospect in sight of a suitable plant for the breeding of birds on a scale sufficiently large for overcoming difficulties which appeared in the course of the work and which will be noted in this paper.

Two preliminary statements concerning this work have been published (Strong, '11 and '12).

Some statements concerning the care of the birds employed and their breeding habits have been included with the hope that they may be of value to other breeders of birds.

Professor Whitman housed portions or all of the stock during several winters, and invaluable suggestions were received from him concerning the care of the doves and methods of bookkeeping. He also furnished some of the stock which was difficult to obtain in the market.

Because of limited cage space Professor Whitman never tried to breed for statistical results. When the newly discovered Mendel's law came into prominence, it naturally received considerable attention from him. Though always critical of Mendelianism, he nevertheless admitted that he had observed phenomena which, at one time, before Mendel's law came to the attention of biologists, seemed to suggest conclusions similar to those of Mendel. However, the apparent absence of character segregations, usually, in the numerous crosses between species as well as varieties of pigeons, which Professor Whitman obtained, did not encourage the growth of Mendelian ideas. Furthermore, his experience was largely with F_1 hybrids as has been the case with other workers in crossing species of birds. Though sceptical of the importance of Mendel's law, he thought it worth while to make a test of it with birds.

Professor Whitman was profoundly impressed with the importance of ancestry and pureness of stock in breeding experiments. As domestic races of animals did not satisfy his ideals in these respects, he naturally turned to wild species for material. Unfortunately for statistical work, wild species do not breed freely in captivity, and hybrids are obtained with difficulty. Standing in a sense between wild species and domestic races of birds in breeding possibilities are the tame ring-doves which are commonly kept in cages. Professor Whitman had found

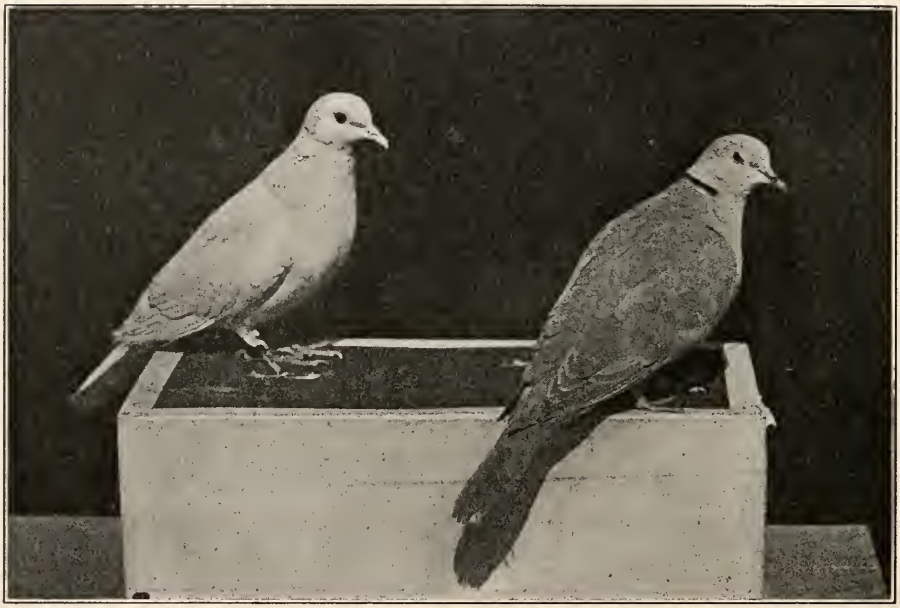


FIG. 1. From a photograph of a male white ring-dove and a female blond ring-dove. The sexes are alike, and hybrids between the blond and white ring-doves are practically not distinguishable from the above. This picture may be used consequently to represent both "pure" and hybrid birds of either sex.

hybrids between the dark or blond ring-dove and the white ring-dove suggesting Mendelian phenomena (see Fig. 1). These birds breed true to type when not crossed, and they have a very simple color pattern. They do well in cages. Variations are so small that a very careful examination under very favorable conditions is required to distinguish individuals.

According to Salvadori ('93), the tame ring-doves are of unknown ancestry, but the dark form is referred to the species *Turtur risorius*, sub-genus *Streptopelia*. In Sharpe's Handlist (Sharpe, '99, p. 78) the term *Streptopelia risoria* Linn. is employed.

Salvadori ('93, p. 415) considered the white ring-dove, *Turtur alba*, to be a white variety of *Turtur risorius*, though he mentions the fact that Temminck and others have regarded the white bird as a distinct species. Whether the white and the blond ring-doves are to be regarded as different species must of course depend on the criterion employed. If the inter-breeding test which is involved in the code of the American Ornithologists' Union is applied, they cannot be called distinct species as they cross freely when together. However, Professor Whitman and others have produced many hybrids between species of doves often very distantly related. Only the freedom with which mating occurs and the apparently perfect fertility of the hybrids can be cited as distinguishing these ring-dove crosses from those between distinct species. The white ring-dove differs from the blond form chiefly in lacking the pigmentation of the latter. The blond bird has a dark bill which is about the color designated by No. 25 in the "Code des Couleurs" of Klincksieck et Valette ('08). The beak of the white bird has a very light flesh-colored tint and is about 071 in the system just mentioned. Both have red feet about 17 in color. The skin of the blond bird is darker than that of the white bird.

Except for the black neck-ring, the predominating colors of the blond dove are fairly well indicated as lying between No. 103A and 103C (see Sharpe, '93, for a detailed description). For practical purposes in this paper, it is sufficient to describe the color of the blond ring-dove plumage as due to a dilute melanin pigmentation. Relatively small numbers of chromatophores appear in the feather germs, and they resemble early stages in the development of the chromatophores which are found in the feather germs of most birds, except that they possess pigment distributing processes. Relatively small and light-colored melanin granules are formed. It is the intention of the writer to discuss these chromatophores more fully in another paper.

Minute traces of pigment are usually to be found in the plumage of the white bird, especially in the rectrices, but the feathers are practically pure white.

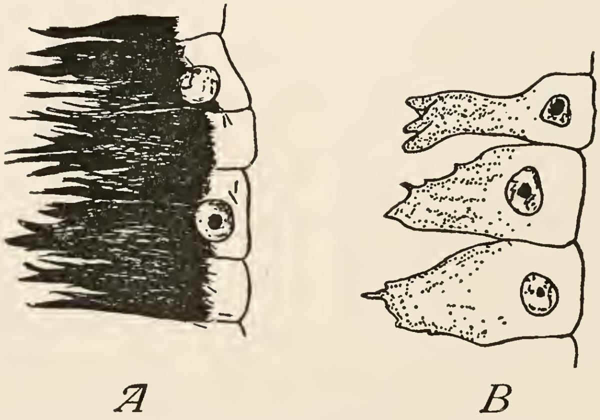


FIG. 2. Cells from the pigment epithelium of the retina, × 1000. The birds were in strong diffused sunlight when killed so that the light condition existed. *A*, blond ring-dove cells; *B*, white ring-dove cells. The peculiar form of the cell processes seems to have been due to the shrinking of the pigment epithelium from the rods and cones layer in the preparations of white ring-dove eyes used for drawing.

The exposed portions of the eyes as seen in the live bird do not differ much in their general appearance for the two forms. An examination of the entire eye removed from its orbit, however, reveals a great difference in pigmentation. The eye of the white bird shows dark pigment in the iris region only, whereas the whole eye ball is dark in the case of the blond bird. A histological comparison was made with the following results. Spherical granules of what appears to be so-called melanin pigment occur in the iris of both birds in considerable quantities so that this region has a black appearance in fixed preparations. These granules vary greatly in size, the largest being about .0005 mm.

in diameter. Most of the granules are smaller than this. The choroid layer in the eye of the blond bird contains large amounts of the same pigment. Only minute traces of pigment occur in the choroid layer of the white ring-dove, and they are found with difficulty. This pigment appears to be the same as that found in the choroid of the blond bird except for its scarcity.

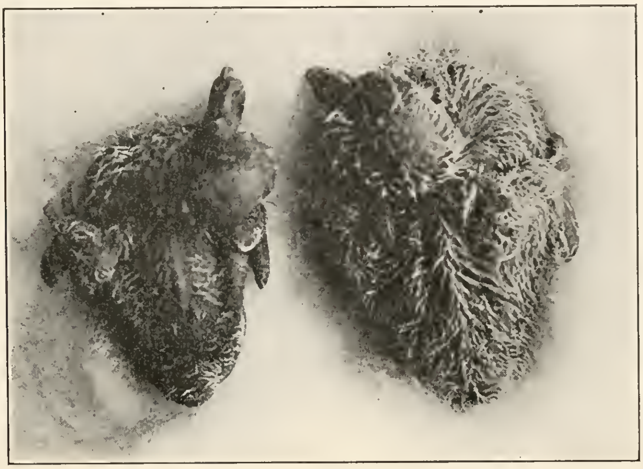


FIG. 3. From photograph of two nestling F_1 hybrids. The smaller bird is two days old, and the larger, three days. The larger bird hatched from the first egg laid, and the smaller from the second egg in the same brood. The contrast in size is largely, if not entirely, due to the difference in age. The larger bird appears exactly like nestlings of pure blond ring-doves of the same age, and the smaller like those of the white ring-dove. The picture fails to show the great contrast in skin color which these birds exhibit at this stage. The smaller bird is very much lighter in color than the blond form, but the photograph failed to show this because the color of the skin has little actinic value.

The pigment epithelium in the eye of the blond bird is richly supplied with slender rod-shaped granules of melanin pigment (see Fig. 2, *A*). These granules are similar in appearance to those which are seen in the feathers of many birds but they differ from those of the ring-dove feather. In the white bird the pigment epithelium of the retina is almost devoid of pigment. A very pale brownish tint is sometimes given to the cell as seen under a high power objective, which is produced by extremely minute granules of what appears to be melanin pigment (see Fig.

2, *B*). These were observed with difficulty when a 1.5 mm. oil immersion objective and a No. 6 Zeiss ocular were employed. The microscopic picture which one of these pigment epithelium cells in the eye of the white bird presents, so far as its contained pigment is concerned, suggests strongly the appearance of a developing feather-germ chromatophore in a very early stage when pigment first appears. Various stages in the development of feather-germ chromatophores are figured by me in another paper (Strong, '02, Plate 6, note Fig. 30, especially).

The nestlings are decidedly different, even at hatching (see Fig. 3). It will be noticed that the down plumage of the white bird is much scantier than that of the blond dove nestling, and it is also somewhat whiter than the yellowish-white down of the blond nestling. The skin color is very different, though the contrast does not appear in the photograph which was used for making Fig. 3. The blond nestling has a rather dark skin which is about No. 93, whereas the white nestling has a light flesh-colored skin about No. 53C. The color of the latter is about that of the beak of the adult white bird.

The blond ring-dove seems to be somewhat more vigorous than the white ring-dove, and its voice is stronger. Professor Whitman was especially impressed with these points of difference. Unfortunately, no precise data for a comparison of the relative vigor of the two forms are at hand, and the above statements are based mostly on general impressions. The comparison for voice is easier to make as this is distinctly different in the two birds.

Melanin pigment appears in the iris region earlier in the blond nestlings than in the white birds. Thus, when the eyes have just become fully open, about one week after hatching, the whole visible eye in the live blond bird appears black. The white ring-dove nestling, on the other hand, shows only a dusky ring about the pupil, and a considerable amount of the eye still appears pink. This phenomenon will be referred to again in connection with the discussion of the observations which have been made by Miss Durham on canaries.

White ring-dove stock is not easy to obtain, and the birds are expensive. It is a curious fact that I found it hard to get female white ring-doves. Some of the stock both of blonds and of whites was imported.

II. Methods and Notes on Breeding Habits.

Mating was accomplished usually by placing birds to be crossed in adjoining cages where other doves could not be seen. Visual impressions seem to be the significant factors in the mating. Other doves may be heard, but little or no attention is paid to them so long as they are not in sight. It was also found advisable to keep the mated pairs where they could not see other doves.

After a few days in the mating cages, a gentle shaking of the wings by both birds usually indicates that a mating has been accomplished, and the two may be placed in one cage with a nest. As males cannot be distinguished from females, with certainty, mistakes are often made in attempts at pairing. At such times, a pseudo-mating may result between males or between two females. Even copulation may take place, and only the appearance of two pairs of eggs or of no eggs at all after a reasonable period of waiting reveals the fact that the birds are not of opposite sex. Such birds readily take other mates when they are placed in cages as described above.

Fertile eggs may be laid in a few days after the birds are placed together. Thus on May 28, 1904, a pair of doves which had been kept in alternate cages for a few days were found to be amorously inclined. They were put in the same cage, and the first egg was laid on the 31st. This egg hatched about 8:30 A.M., June 15. The second egg was laid on June 2, and it hatched about 8:30 A.M., June 16. There was no reason to believe that the female had been fertilized by another male before the mating was begun. On June 23, the young doves were observed rising on their feet in the nest and elevating their wings. Their eyes were open on this date. These nestlings left the nest on the 27th, and one was observed sitting on a perch a few inches above the floor of the cage on June 28. Both were seen feeding from the seed dish used by their parents on the 30th. The plumage at this time was well developed except about the bill as is characteristic of young pigeons. The feathers of the bill region develop after the birds are weaned.

It was my experience that adult ring-doves more than one year old may begin breeding, when kept in a heated building, in late January or early February. Breeding operations, even under apparently favorable conditions, may be delayed until May, especially with young birds. The greatest number of young produced in a season by a single pair of ring-doves was nine from five sets of two eggs each (see mating 8, Table XXI.). The records for this pair were as follows.

Brood 1.—First egg laid April 12, 1905. Hatched April 28. Second egg laid April 14. Did not hatch.

Brood 2.—First egg laid May 15. Hatched May 31. Second egg laid May 17. Hatched May 31.

Brood 3.—First egg laid June 24. Hatched July 10. Second egg laid June 26. Hatched July 10.

Brood 4.—First egg laid Aug. 4. Hatched Aug. 20. Second egg laid Aug. 6. Hatched Aug. 21.

Brood 5.—First egg laid Oct. 5. Hatched Oct. 21. Second egg laid Oct. 7. Hatched Oct. 22.

With the first three broods, the eggs both hatched in the early morning of the fourteenth day after the second egg was laid. It will be noticed that there was some delay in the hatching of the eighth and tenth eggs as is apt to be the case at the end of the season. Both young of brood 4 died during the first week of September. Under favorable conditions, an average of six young per season may be considered good. Delays due to sickness, errors in mating, and occasional deaths reduce the average which might otherwise be higher. It will be seen in Table XXI. that there is a large variation in the number of eggs which are laid in a season.

The breeding habits of the blond ring-dove have been described by Whitman ('98), and in more detail by Craig ('08 and '09).

III. Results.

All of the hybrids which were obtained resembled one or the other of the parents. The dark hybrids had, however, a slight tendency to be somewhat lighter in color than the dark or blond parent. According to Professor Whitman, the voice of the hybrids tends to be intermediate. A comparison of the voices of many individuals is not easy to make, and I never felt certain enough about this point to venture an opinion one way or the other. The white birds often show slight traces of melanin pigment in their feathers, but this was also observed in presumably pure stock as has been noted. The sex of ring-doves can be determined satisfactorily only by breeding tests or by dissection. The sex of a number of individuals was not ascertained as these died when there was no one on hand to make the necessary dissection. Some nestlings have also been included, and their sex was not determined.

The total number of hybrids hatched was 151, and the sex of 74 of these was ascertained. Of these, 32 were males and 42 were females. This preponderance of females is of no significance. Table I. gives results of crosses between blond males and white females. The ordinary Mendelian expectation is realized for color here, as all of the offspring in F_1 are essentially like one parent in color, and the blond coloration is dominant. Whiteness of plumage or absence of pigmentation is recessive to the presence of pigment. Eight out of the ten birds of known sex were males.

TABLE I.

BLOND MALES × WHITE FEMALES. MATINGS 1, 3, 4, 17, AND 23.

F_1 14 offspring all dark. 8 males, 2 females, 4 sex ?.

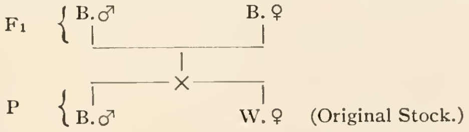
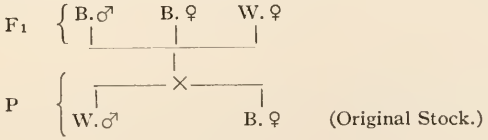


TABLE II.

WHITE MALES × BLOND FEMALES. MATINGS 2, 5, 16, 26, 29, 30, AND 33.

F_1 18 blonds: 7 males, 3 females, and 8 sex?.
23 whites: 13 females and 10 sex ?.



With the reciprocal cross as in Table II., about one half of the offspring were white in F_1, and *all of the white birds whose sex was ascertained were females.* Again the dark hybrids were mostly males.

As only four blond female hybrids and no male white hybrids were obtained in F_1, it was not possible to do much breeding of hybrids *inter se*. Two pairs of blond hybrids were mated and the results of their breeding are given in Table III. The occurence of a white bird in F_2 is of course to be expected on a Mendelian basis. Again the same preponderance of males appears.

TABLE III.

BLOND F_1 HYBRID MALES × BLOND F_1 HYBRID FEMALES. MATINGS 28 AND 44.

F_2 10 blonds: 3 males, 1 female, and 6 sex ?.
1 white female.

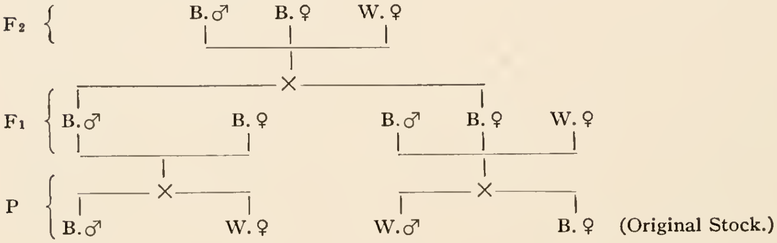
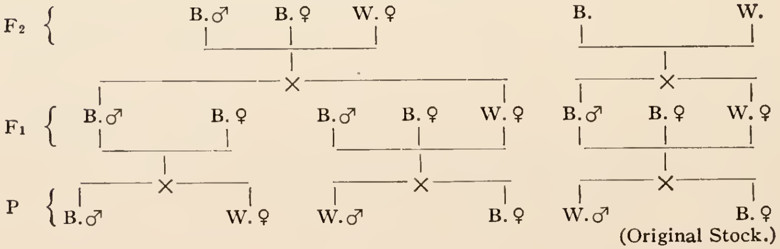


TABLE IV.

BLOND F_1 HYBRID MALES × WHITE F_1 HYBRID FEMALES. MATINGS 14, 20, 35, 37, AND 42.

F_2 6 blonds: 1 male, 2 females, 3 sex ?.
5 whites: 3 females, 2 sex ?.



Blond hybrid F_1 males when mated with white hybrid F_1 females (see Table IV.) gave results which are similar to those obtained in Table II. where approximately equal numbers of white and blond birds occurred. Again the white birds sexed were all females.

Professor Whitman obtained similar results which were given to Bateson and are referred to by the latter in a footnote (Bateson, '09, p. 194). Through the courtesy of Mrs. Whitman, I am permitted to give exact statistics as copied from Professor Whitman's records. These were known in a general way to me, having been discussed in conversations before his death, and they are as follows: when white male ring-doves were crossed with dark female ring-doves 5 blonds and 4 whites were obtained. White male ring-doves crossed with females of the very distinct species, *Turtur humilis*, produced five dark birds and twelve whites. In both crosses the white offspring were all females and the dark hybrids were all males "so far as was known."

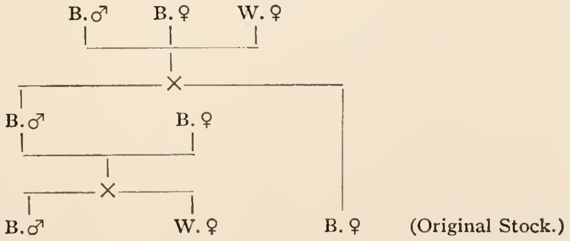
There being no white F_1 male hybrids, it was impossible to get F_2 offspring from the white hybrids, and the white hybrids were consequently crossed back upon the original white stock (see Table VII.). All of the offspring of this cross were white, and males as well as females appeared. A white male was also obtained in a cross between a blond male and a white female both birds being the offspring of hybrids crossed back on the original stock (see Table XII.).

A blond hybrid male which had been obtained by crossing back was mated with a pure blond female, and all of the five offspring were blonds. Three of the four birds whose sex was learned were females. The effects upon the sex ratio of crossing back upon the original stock will be discussed later in this paper. Other results are given in Tables V., VI., VIII.–XI. and XIII.–XVII.

In looking over these results, it is quite apparent that white-

TABLE V.

BLOND F_1 HYBRID MALE (TABLE I.) × BLOND FEMALE. MATING 22.
Offspring 9 blonds: 5 males, 1 female, 3 sex ?.
5 whites: 4 females and 1 sex ?.



ness of plumage behaves as a sex-linked character in the crossings which are described in this paper. Associated with this whiteness are the pigmentation phenomena of the eye, and the scantier

TABLE VI.

WHITE MALE × BLOND F₁ HYBRID FEMALE (TABLE I.). MATING 10.

Offspring 2 blonds: 1 male and 1 sex ?.
1 white: sex ?.

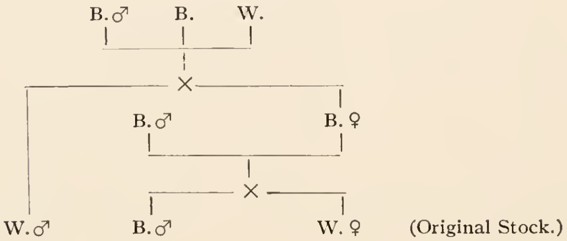

B.♂
B.
W.
×
B.♂
B.♀
×
W.♂
B.♂
W.♀
(Original Stock.)

TABLE VII.

WHITE MALES × WHITE F₁ HYBRID FEMALES (TABLE II.). MATINGS 8 AND 9.

Offspring 18 whites: 2 males, 3 females, 13 sex ?.

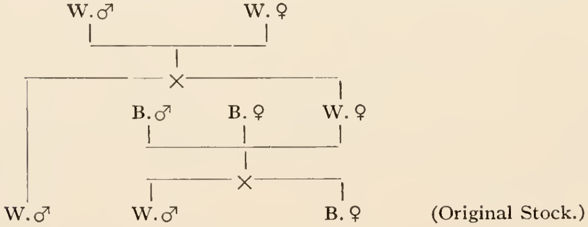

W.♂
W.♀
×
B.♂
B.♀
W.♀
×
W.♂
W.♂
B.♀
(Original Stock.)

TABLE VIII.

BLOND MALE × WHITE HYBRID FEMALE (TABLE V.). MATING 32.

Offspring 3 blonds: 2 males and 1 sex ?.

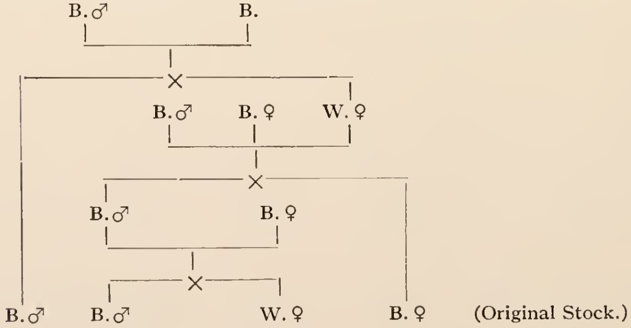

B.♂
B.
×
B.♂
B.♀
W.♀
×
B.♂
B.♀
×
B.♂
B.♂
W.♀
B.♀
(Original Stock.)

TABLE IX.

BLOND HYBRID MALE (TABLE V.) × BLOND FEMALES. MATING 31.

Offspring 5 blonds: 1 male, 3 females, 1 sex ?.

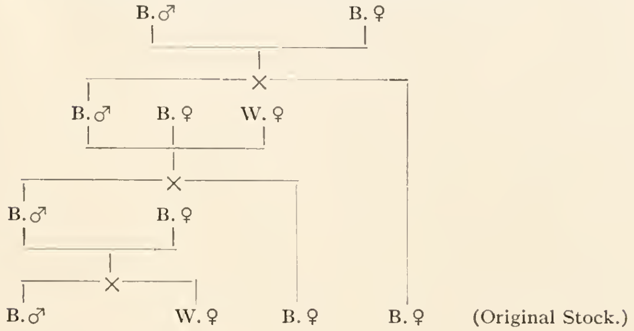
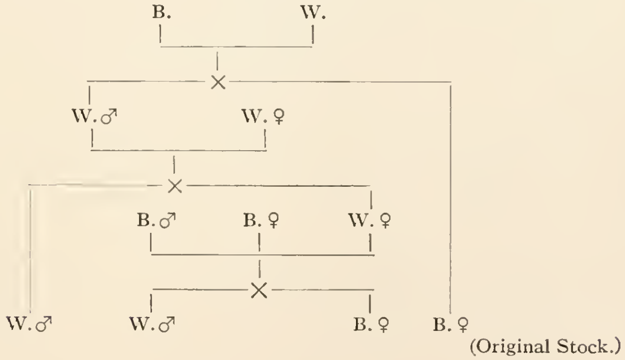


TABLE X.

WHITE HYBRID MALE (TABLE VII.) × BLOND FEMALES. MATING 12.

Offspring 6 blonds: sex ?.
1 white: sex ?.



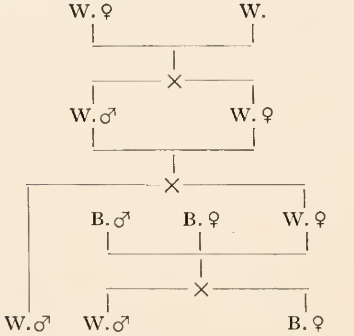
nestling down already described on pp. 296–8. These characters and doubtless others not noticed act as a group, and they appear in female birds of the F_1 hybrid generation when the male bird possesses them. The characters of the blond bird come very near being sex-linked as they appear in only a few female hybrids.

There are many interesting points of resemblance between the results of these experiments and those obtained by some other workers. Apparently all of the cases of sex-linked inheritance in birds involve similar phenomena. Strikingly similar results

TABLE XI.

WHITE HYBRID MALE (TABLE VII.) × WHITE HYBRID FEMALE (TABLE VII.).
MATING 13.

Offspring: 3 whites: 1 female and 2 sex ?.

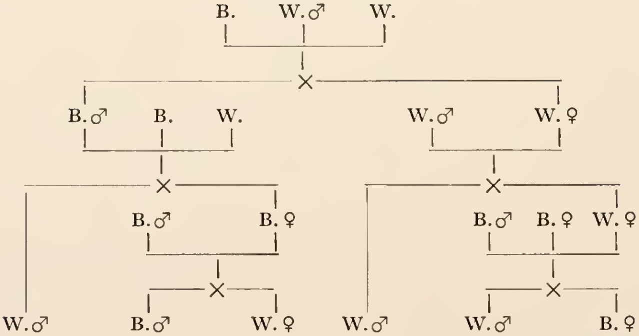


(Original Stock.)

TABLE XII.

BLOND HYBRID MALE (TABLE VI.) × WHITE HYBRID FEMALE (TABLE VII.).
MATING 15.

Offspring: 2 blonds, sex ?.
4 whites, 1 male and 3 sex ?.



(Original Stock.)

are those which were obtained by Durham and Marryat ('08) with canaries. Their cinnamon or pink-eyed canaries correspond in breeding behavior to the white ring-doves and their green or black-eyed canaries to the blond ring-doves. The results differ in that the ring-dove hybrids lack the variability which was characteristic of the canaries—a point of apparently no significance from the standpoint of the problems which are under discussion. No crossing comparable to their mating

TABLE XIII.

BLOND HYBRID MALES (TABLE I.) × WHITE HYBRID FEMALES (TABLE V.). MATINGS 39 AND 41.

Offspring: 6 blonds: 1 male, 3 females, 2 sex ?.
1 white female.

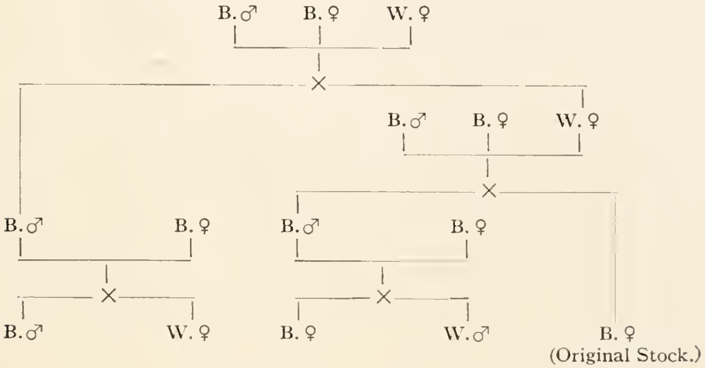


TABLE XIV.

BLOND HYBRID MALE (TABLE V.) × BLOND HYBRID FEMALE (TABLE I.). MATING 40.

Offspring: 2 blonds, sex ?.

TABLE XV.

BLOND HYBRID MALE (TABLE V.) × WHITE HYBRID FEMALE (TABLE II.). MATING 43.

Offspring: 1 blond, sex ?.

TABLE XVI.

BLOND HYBRID MALE (TABLE I.) × WHITE HYBRID FEMALE (TABLE XI.). MATING 21.

Offspring: 2 blonds, sex ?.

TABLE XVII.

WHITE HYBRID MALE (TABLE XII.) × WHITE HYBRID FEMALE (TABLE VII.). MATING 19.

Offspring: 1 white female.

TABLE XVIII.

BLOND MALES × BLOND FEMALES. MATINGS 24, 27, AND 38.

Offspring: All blond: 4 males, 5 females, 7 sex ?.

(Other matings of blond stock birds were made, and the offspring were also all blonds. They are not included here because exact records were not kept for them.)

TABLE XIX.

WHITE MALE × WHITE FEMALE. MATING 25.

Offspring: All white: 1 male, 3 white females, 3 sex ?.

No. 5 was attempted with the ring-doves because of a lack of white stock females. At the time the ring-dove matings were made, the work of Durham and Marryat was not known to the writer.

In all of the parallel cases (and these include some forms outside of the bird group), when the male parent has the dominant character, the offspring in F_1 resemble the male parent and few or no females occur as a rule. When in the reciprocal cross the male bird is recessive, the offspring in F_1 are about equally males and females; the recessive characters appear *only in female offspring.*

The sex-ratio for F_1 offspring bearing the dominant characters varies greatly in different combinations. In both the canaries and the ring-doves the number of dominant females is very small and the proportion seems to be about the same in both. In some other combinations, as in the case of the crossings which were made by Pearl and Surface ('10*a*) between Barred Plymouth Rock males and Cornish Indian Game females, about equal numbers of males and females had the dominant barring. *No females* were barred in the reciprocal cross, however.

Other essentially similar cases are given in the following table where the dominant form is placed before the recessive. The famous experiments with currant moths described by Doncaster and Raynor ('06) could be included in this list if *laticolor* males occurred in nature.

TABLE XX.

(F_1 females have dominant characters only when the male parent has them, in the following combinations:)

Crossings.

Brown Leghorn × Silky fowl. Bateson ('09 and '11).
Black-red Game Bantam × Brown-red Game Bantam. Hagedoorn ('09).
White Rock × Brown Leghorn. Goodale ('10).
White Wyandotte × Brown Leghorn. Sturtevant ('11).

Dark Brahma × Brown Leghorn. Lacing characters. Davenport ('12).

Barred Rock × Langshan, Morgan and Goodale ('12).

[In the above table I have classed the Silky fowl as recessive to the Brown Leghorn for the peculiar pigmentation which is characteristic of the Silky fowl mesodermal tissues. With this interpretation the case furnishes, in my judgment, a strong argument for the position taken in this paper as it does not seem probable that the pigmentation which appears in F_1 females is derived from the Brown Leghorn hen.]

The commonly employed gametic analysis of these phenomena involves the assumption that in such cases, at least, the female is heterozygous for sex and the male homozygous. The female of the dominant form is assumed to be heterozygous also for the somatic characters involved. To various writers it has seemed more feasible to either ignore the opposing cytological evidence with regard to sex determination or to assume that the cytological conditions are different in these forms. Unfortunately, cytological evidence is difficult to obtain with birds, though Guyer ('09) has published observations which indicate that the common fowl has two kinds of sperms.

Other interpretations have been suggested by de Meijere ('11). who assumes that potentialities of both sexes are present in each gamete. According to de Meijere, one sex gets the upper hand in the egg during fertilization at the expense of the other. Just how this writer conceives of the operation in the phenomena of sex-linked inheritance, is not clear to me.

In thinking over these phenomena, I have been much impressed with the fact that *recessive characters appear in F_1 only when the male parent is recessive*, and it is hard to resist forming the opinion that the recessive male is responsible for the situation. In the case of the turtle dove genus, whenever a white ring-dove male is crossed with a female blond ring-dove or with a female of *Turtur humilis*, white female offspring occur in F_1. As this paper is going to press, an article by Staples-Browne ('12) has appeared in which crossing experiments with pigeons are described. These include crosses between "Turtle doves" (*Turtur turtur*) and so-called "White Java Doves" which I infer to be the white ring-doves of this paper. The results seem to be similar to those which were obtained by me, so far as they go. Two matings between "White Java" males and female "Turtle doves" were made and ten offspring were obtained—four "dark males," four "dark, sex uncertain" and two white females.

Staples-Browne also crossed the blond ring-dove (called "Barbary Dove" in his paper) with his "White Java" doves and obtained results which are like those described by me in this paper, except that in two matings between male blonds and white females, three male and three female white birds appeared in F_1. The explanation of these results given by Staples-Browne, that the male parents in these two cases were hybrids is undoubtedly correct. He states that much difficulty was experienced in obtaining pure "Barbary" stock.

Records are thus at hand of the appearance in F_1 of white birds, whenever a white male ring-dove is crossed with females of at least two Linnæan species and also with the blond ring-dove. The assumption that the females of all of these three species, *Turtur risorius*, *T. humilis* and *T. turtur* are heterozygous for color seems to me more difficult to accept than the idea that the sperms of the male white ring-dove are responsible for the F_1 white birds.

According to the interpretation of these cases of sex-linked inheritance which has been advanced by Spillman ('08), Bateson ('09) and others, the following two assumptions may be made in addition to those already stated (see Bateson, '09, p. 175): "That when in F_1 the two dominant characters femaleness and the 'somatic' factor co-exist, there is spurious allelomorphism or repulsion between them, such that each gamete takes one or other of these factors, not both." The following scheme though familiar is repeated here for the sake of clearness in making a comparison with the interpretation which I am about to propose.

D = dominant. R = recessive.

1. Dominant ♂	×	Recessive ♀.
composition DD ♂♂		RR ♀♂
gametes all D ♂		R ♀
		R ♂

F_1 *dominant males.*		dominant females.
DR ♂♂		DR ♀♂
2. Recessive ♂	×	Dominant ♀.
composition RR ♂♂		DR ♀♂
gametes all R ♂		R ♀
		D ♂
F_1 *dominant males.*		*recessive females.*
DR ♂♂		RR ♀♂
3. Dominant F_1 ♂	×	Recessive ♀.
composition DR ♂♂		RR ♀♂
gametes D ♂		R ♀
R ♂		R ♂
dominant males.		*dominant females.*
result DR ♂♂		DR ♀♂
recessive males.		*recessive females.*
RR ♂♂		RR ♀♂
4. Dominant F_1 ♂	×	Dominant ♀.
composition DR ♂♂		DR ♀♂
D ♂		R ♀
gametes R ♂		D ♂
dominant males.		*dominant females.*
result DD ♂♂		DR ♀♂
and also		*recessive females.*
DR ♂♂		RR ♀♂

If, however, the female is regarded as homozygous for sex and also for somatic characters, and the male as heterozygous for sex, these phenomena may be explained in my judgment with the following additional assumption: that the female producing sperms of the recessive male have the power of suppressing or checking the development of dominant characteristics which may be carried by the egg or it may be that these sperms lack something which is necessary for the development of the dominant characters. This position is supported by all of the cytological evidence known to me, and it is not inconsistent with the idea that potentialities of both sexes may be present in both male and female gametes as well as in zygotes if such is the case. The castration experiments of Goodale ('10), for instance, have demonstrated that some female birds at least may develop male characteristics when their ovaries are removed. The processes involved in determining the sex which dominates the organism may be the same whether one or both sexes are represented in it. So-called "spurious allelomorphism" is not assumed in this scheme.

This alternative interpretation may be applied to the ring-doves as follows. The female determining gamete is designated by the letter $_f$ as a subscript with an additional letter $_w$ in the case of the white male bird. All male gametes are designated by the male sign and female signs are used for the female gametes. These symbols are used thus with the hope that the formulæ may be more easily read, and also because it does not seem to the writer to be very appropriate to designate sperms, for instance, by both male and female signs as is commonly done when the male is regarded as heterozygous or to call eggs male and female as is done when the female is considered as heterozygous for sex. Both sexes are represented under the head of composition because the zygote is the result of a union of male and female gametes. Characters which are recessive to others in the same individual are included in parenthesis. In the case of F_1 females, the $_w$ effect is interpreted as changing B to W.

1. Blond ring-dove male	×	White ring-dove female.
composition B ♂ B (♀)		W ♀ (W ♂)
producing gametes B ♂ B_f ♂		W ♀ W ♀
F_1 *Blond males.*		*Blond females.*
B ♂ (W ♀)		B ♀ (W ♂)

2. White ring-dove male	×	Blond ring-dove female.
composition W ♂ (W ♀)		B ♀ B (♂)
producing gametes W ♂ W_{fw} ♂		B ♀ B ♀

F_1 *Blond males, white females, and occasionally blond females.*

B ♂ (W ♀) W ♀ W (♂) B ♀ (W ♂)

That the females in mating No. 2 are mostly white is explained by the assumption already made that the female determining gametes (subscript $_f$) of the male white bird are responsible for the absence of pigment. The $_w$ effect may or may not be connected with an accessory chromosome.

In the case of the canaries, Durham and Marryat found that their formula (*i. e.*, that given on pp. 310–1 of this paper) did not explain the occurrence of two F_1 "black-eyed" females when the male bird was pink-eyed. The same difficulty appears with the ring-doves as may be seen on comparing Table II. where it will be seen that three blond F_1 females appeared. The difficulty is no greater in the scheme just presented in this paper, and it would seem to be due possibly to the occasional failure of the $_{fw}$ sperm to produce the $_{w}$ effect.

It may be noted also that neither scheme explains the great excess of F_1 males in the ring-dove and canary experiments. The occurrence of a preponderance of males is a common and well known result of crosses between bird species, for which no satisfactory explanation has been given. It is of course conceivable that female-determining sperms may in such cases encounter difficulties in the egg of another species which may be great enough to prevent the development of femaleness, more or less frequently. (See Guyer, '09*a*.)

The gametic analysis is carried still further as follows, and it may be noted that corresponding matings bear the same numbers.

3. Blond F_1 ring-dove male × White ring-dove female.
composition B ♂ (W ♀) W ♀ W (♂)
B ♂
B_f ♂
producing gametes W ♂ all W ♀
W_{fw} ♂
result Blond males. *Blond females.*
B ♂ (W ♀) B ♀ (W ♂)
White males. *White females.*
W ♂ W (♀) W ♀ W (♂)

See Table XII. of this paper where the birds crossed are probably potentially the same as the forms which are implied by this mating.)

4. Blond F_1 ring-dove male × Blond ring-dove female.
composition B ♂ (W ♀) B ♀ B (♂)

B ♂
producing gametes B_f ♂ all B ♀
W ♂
W_{fw} ♂
result Blond males. *Blond females.*
B ♂ B (♀) B ♀ B (♂)
and also *white females.*
B ♂ (W ♀) W ♀ W (♂)

(See Table V. of this paper.)

Still another mating combination is necessary to express the conditions which may be expected when F_1 blond ring-dove hybrids are mated *inter se* for the production of F_2 offspring.

5. Blond F_1 hybrid male × Blond F_1 hybrid female.
composition B ♂ (W ♀) B ♂ (W ♀)
B ♂
producing gametes B_f ♂ B ♀
W ♂ W ♀
W_{fw} ♂
F_2 *Blond males.* *Blond females.*
B ♂ B (♀) B ♀ B (♂)
and also and also
B ♂ (W ♀) B ♀ (W ♂)
White males. White females.
W ♂ W (♀) W ♀ W (♂)

(See Table III. of this paper.)

From the two matings of this sort made, only one, out of the eleven F_2 offspring obtained, was white, whereas the expectation would be one out of four. However, the number of F_2 offspring was too small to make a critical test and this result is not especially significant.

It will be observed that white hybrids crossed back on white stock and white hybrids crossed *inter se* (see Tables XI. and XVII.) gave white offspring, and males appeared as is to be expected. The white hybrids are so-called extracted recessives.

Other results were also obtained some of which have been mentioned on pp. 299–300 of this paper. In Table XXI., the following records may be found:

1. The number of broods in a season.
2. The number of eggs in a brood.

3. The eggs hatching.
4. The period of incubation for each egg, when known.
5. The sex and color of the nestling hatching from each egg.

Table XXI.

B., blond; W., white; K., died in egg; N. D., no development; ?, records uncertain.

Matings.	Brood 1.	Brood 2.	Brood 3.	Brood 4.	Brood 5.
(1904) 1	19B.; K.				
2	16W. ♀; 15W. ♀	K.; 16W.	Eggs small. N.D.; N.D.		
3	15B. ♀; 14B.				
4	N.D.; 14-B.	(August) 15-B.; 13-20B.	(September) N.D.; N.D.	(October) N.D.; N.D.	
5	16B.♂; no 2d egg	16B.♂; 14B.			
(1905) 8	16W.; K.	16W.; 14W.♂	16W. ♀; 14-W.	16W.; 15W.	16W. ♀; 15W.
8	W.; W. March, 1906				
9	15W.♂; 14W.	16W.; 14W.	14-12W. ♀; 14-W.	(November) 17W.; N.D.	
10	15B.♂; 14B.	16W.; K.			
(1906) 12	16B.; 14B.	16B.; 15B.	K.; 14B.	15B.; 14W.	
13	N.D.; N.D.	15W. ♀; 14W.	?; ?		
14	16B.; N.D.	?; W.	?; ?		
15	15W.; 14W.	15W.; 14W.♂	15B.; 14B.		
16	15B.; 14W.	15W.; 14.	?; ?	(October) N.D.; N.D.	(November) N.D.; N.D.
17	15B.♂; 14B.♂	15B.♂; 14B.♂	?; ?		
(1907) 19	16W. ♀; 14	15; K.	K.; K.		
20	K.; 0	15B.; N.D.			No breeding after June in 1907.
(1908) 22	W. ♀; W.	B.♂; B.♂	B.♂; W. ♀	W. ♀; W. ♀	
(1909) 22	15B.♂; 14B.	B.♂; 0	14-B. ♀; 13-B.♂	15B.; N.D.	
(1908) 23	B.♂; N.D.	B.♂; B.♂	B.♂; B. ♀		
24	B. ♀; B. ♀	B. ♀; B.♂	B. ♀; B.♂	B. ♀; B.♂	
25	W.; W.	W. ♀; W.♂	15W. ♀; 14W. ♀	W.; K.	
26	W. ♀; K.	W. ♀; W. ♀	15W. ♀; 14B.♂		
(1909) 26	16B.; 16W. ♀	W. ♀; W.	16W.; 14W.	W.; W.	
27	N.D.; 14B.♂	B.; 0			
28	15B.; 14B.	16B.; K.	15B.; 14B.♂	B. ♀; W. ♀	
29	26B.; 15B.	B. ♀; B.	15B. ♀; K.	15B.♂; K.	
30	W.; W. ♀	15W.; 14B.	B.♂; B.♂		
31	B. ♀;	15B.; 14B.♂	15B. ♀; B. ♀		
32	14B.; 13-B.♂	15B.♂; N.D.			
33	15W. ♀; B.	15W. ♀; 14W. ♀			
35	15W. ♀; 14B. ♀	15B.; 14W.			
37	15B. ♀; 14B.♂				
38	15B.; 14B.	16B.; 14B.	15B.; 14B.		
39	15B. ♀; 0	15B. ♀; 14B. ♀			
40	15B.; 14B.				
41	15B.♂; 14	W. ♀; B.			
42	15W. ♀; 14W. ♀				
43	B.; N.D.				
44	B.♂; B.	15B.♂; 15B.			

In the above table the period between laying and hatching is given, when known. In some cases, as in mating 4, brood 2, this period is indicated in both days and hours. When no second egg was laid, a zero appears. Both birds were stock blonds in matings 24, 27, and 38. Stock whites were used in mating 25.

Table XXII.

Matings.	See Table.	Total Eggs Laid.	Total Eggs Hatched.	Total Offspring.					
				Blond.			White.		
				♂♂	♀♀	Sex?	♂♂	♀♀	Sex ?
1	I.	2	1						
2	II.	6	3					2	1
3	I.	2	2		1	1			
4	I.	8	3			3			
5	II.	3	3	2		1			
8	VII.	10	9				1	2	7
8 (1906)	VII.	2	2						2
9	VII.	8	7				1	1	4
10	VI.	4	3	1		1			1
12	X.	8	7			6			1
13	XI.	6	3					1	2
14	IV.	6	4			1			1
15	XII.	6	6			2	1		3
16	II.	10	6	1		1		1	2
17	I.	6	6	4					
19	XVII.	6	3			1		1	
20	IV.	3	1			1			
21	XVI.	2	2			2			
22	V.	8	8	3	5			4	1
22 (1909)	V.	8	6	3	1	2			
23	I.	4	4	4	1				
24	XVIII.	8	8	3	5				
25	XIX.	8	7				1	3	3
26	II.	8	5	1				4	
26 (1909)	II.	8	8			1		2	5
27	XVIII.	5	2	1		1			
28	III.	8	7	1	1	4			
29	II.	8	6	1	2	3			
30	II.	6	6	2		1		1	2
31	IX.	5	5	1	3	1			
32	VIII.	4	3	2		1			
33	II.	4				1		3	
35	IV.	4	4		1	1		1	1
37	IV.	2	2	1	1				
38	XVIII.	6	6			6			
39	XIII.	3	3		3				
40	XIV.	2	2			2			
41	XIII.	4	4	1		2		1	
42	IV.	2	2					2	
43	XV.	2	1			1			
44	III.	4	4	2		2			
Totals		218	174	34	19	48	4	30	36

As the first egg laid was regularly marked, it was possible to know which egg hatched first. In Table XXI., the first egg laid appears before the other. The first nestling to hatch was marked by a clipped toe. Aluminum ring-band tags were placed on one leg of each bird before it left the nest.

Some of these data are given in other form in Table XXII. where each mating is referred to the table which describes it.

The periods between laying and hatching may be summarized as follows in Table XXIII.

Table XXIII.

		Number of Individuals.		
	Period.	Stock.	Blond Hybrids.	White Hybrids.
First egg.	15 days.	5	26	12
" "	16 "	2	8	9
" "	17 "			1
" "	19 "		1	
Second egg.	14 days.	5	22	14
" "	15 "		3	3
" "	16 "			
" "	18 "			

It will be noticed that no cases of more than two eggs in a clutch occurred.

The idea has existed that the first eggs in a pigeon clutch usually give rise to males, though I know of no studies which would support this idea. An inspection of the data in Table XXI. proves this assumption to be without foundation with ring-doves at least. A summary of the results from those matings where the offspring were not mostly either males or females is given below. The matings from which the statistics were taken are as follows: Nos. 2, 8–10, 15, 16, 22, 24–33, 35, 37–40, 42 and 44.

First egg	Male	13
" "	Female	28
Second egg	Male	15
" "	Female	15

It is obvious that ring-doves, at least, do not show any correlation between sex and the order of laying or of hatching. Cole ('11) obtained similar results with tumbler pigeons.

IV. Summary.

1. Hybrids between blond male ring-doves and white female ring-doves are all blonds and they are mostly males.

2. The offspring of the reciprocal cross are about equally blonds or whites, but *all of the white birds are females.* Whiteness and the characters associated with it are sex-linked. Al-

most all of the blond birds are males. Male white birds appear, however, when white hybrid females are crossed back on white stock males.

3. The nestling hybrids are identical in appearance with the nestlings of the corresponding blond and white uncrossed ring-doves.

4. The phenomena observed are remarkably similar to those described by Durham and Marryat ('08) for canaries.

5. Sex-linked inheritance in birds and elsewhere also can be explained, in my judgment, more logically with the assumption that the male is heterozygous for sex and the female homozygous, than by the contrary hypothesis.

6. The appearance of recessive characters in F_1 when the male parent is recessive may be explained with the assumption that the female determining gametes of the male parent may either possess or lack something which is responsible for the absence in female offspring of dominant characters carried by the female parent.

7. No evidence was obtained in support of the old idea that the first egg laid by doves produces a male.

8. Observations concerning the period of incubation and other points in the breeding habits of ring-doves are described.

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WEAK PARTHENOGENETIC RACES OF HYDATINA SENTA SUBJECTED TO A VARIED ENVIRONMENT.

D. D. WHITNEY.

Some years ago Weismann maintained that the unicellular organisms were not subject to natural death but were immortal. No individual died from old age but in reproduction went to form the offspring. Later Maupas made some observations upon pedigreed cultures of certain of the protozoa and found that, although the individuals did not develop senile decay, nevertheless, the race did go gradually into senile decay and died out if no conjugation was allowed. Later Calkins, Woodruff, Gregory, and others confirmed Maupas's observations but, in addition, Calkins and Woodruff have found that when the races were very weak and near the point of death they could be artificially stimulated and restored to their former vigor by various substances in the food solution. After which they were able to reproduce for many more generations before becoming weak again and then could be restimulated again. However, there always came a time when nothing would reinvigorate the races and they consequently died out. Recently Woodruff has shown that certain races of paramœcia never become weak provided the environment is more or less varied.

In recent papers Whitney has shown that the rotifer, *Hydatina senta*, can be propagated parthenogenetically for several hundred generations but each race gradually becomes weaker and weaker and finally dies out. However, when they are in this weak condition the races may be restored to the normal degree of vigor by cross-breeding. Close-breeding within each race only slightly restores their vigor.

At the time many experiments were made by changing the environment in order to determine whether any external influence would restore these weak races to their normal vigor as had been done in the weak races of protozoa.

The two weak races, *A* and *B*, which have been fully described in a former paper were used in these experiments. The criterion of weakness was the rate of reproduction. Races *A* and *B* in the spring of 1911 were allowed to produce close-fertilized eggs in the 370th and the 380th parthenogenetic generations respectively. From these close-fertilized eggs females developed which reproduced parthenogenetically for a time but by the end of the summer they had been allowed to close-breed three or four times. At this period most of the experiments described in this paper were undertaken.

Ever since the two races were started in the fall and winter of 1908 and 1909 they have been subjected to a very constant environment. They have been kept at room temperature ranging from 18° C. to 22° C. and always have been in a food solution of horse manure. During the last sixteen months of their parthenogenetic propagation they were even fed upon a pure culture of the flagellate, *Polytoma*, which was reared in a horse manure solution. A certain quantity of this horse manure solution containing the protozoa was added to a certain amount of tap water and placed in syracuse watch glasses thus making the amount and concentration of the food culture water in which the rotifers lived practically constant.

Different Food Materials.

In order to cause a great variation in the food factor and also of the chemicals in the water food cultures were prepared from the feces of various herbivorous, carnivorous and omnivorous animals. These food cultures were made in battery jars and inoculated with a miscellaneous lot of protozoa from several small fresh water ponds. The rotifers were placed in these large jars and allowed to live freely from 9 to 19 days and then were transferred to other food jars which contained feces of a different animal. In this manner a great variation of protozoa and of chemical substances in the water were obtained. These experiments extended through about three months. Table I. shows the data obtained at the end of the experiments which demonstrate that no reinvigoration had taken place in either of the two weak races.

TABLE I.

Table showing the comparative reproduction rates of various weak races after they had been subjected to culture waters, from 9–19 days, which were made from the following feces: horse, Sept. 18–27; guinea-pig, Sept. 27 to Oct. 9; man, Oct. 9–23; dog, Oct. 23 to Nov. 10; sheep, Nov. 10–25; hen, Nov. 25 to Dec. 14; horse, Dec. 14——. These comparative reproduction rates show that the general vigor of the weak races was not restored to that of the normal race (control) by the varied environment.

Experiment.	Time, 1911.	Control. Race *A* Between 470–500 Parthenogenetic Generations.			*A* 3d. (Race *A* Close-fertilized 3 Successive Times After the 370th Parthenogenetic Generation, March–September.)			*B* 4th. (Race *B* Close-fertilized 4 Successive Times After the 380th Parthenogenetic Generation, March–September.)			Control. Race *C* at the 390th Parthenogenetic Generation.			Control. Parthenogenetic Race *F* Developed from a Wild Fertilized Egg November 22, 1911.			Generations.
		Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	
1	Eve. 12–14 Eve. 12–17	6	1	0.16	2	1	0.5	4	2	0.5	8	44	5.5	8	87	10.87	1
	Eve. 12–18 Eve. 12–21	9	6	0.66	7	29	4.14	8	11	1.37	8	42	5.25	9	97	10.77	2
	Eve. 12–21 Eve. 12–24	4	4	1	10	22	2.2	9	17	1.88	10	49	4.9	8	69	8.62	3
2	Eve. 12–16 Eve. 12–19	5	5	1	1	4	4	1	2	2	5	44	8.8	3	42	14	1
	Eve. 12–19 Eve. 12–22	8	3	0.37	9	25	2.77	9	5	0.55	8	19	2.37	9	51	5.66	2
	Eve. 12–22 Eve. 12–25	10	3	0.3	10	25	2.5	9	10	1.11	10	32	3.2	8	56	7	3
	Summary	42	22	0.52	39	106	2.71	40	47	1.17	49	230	4.69	45	402	8.93	

REST.

It is sometimes suggested that a period of inactivity will stimulate weak organisms. Therefore both races were kept at 3° C. to 7° C. for about three weeks in a rich food culture and then were placed at room temperature but failed to show any increase in the rate of reproduction. Some close-fertilized eggs of race *B* were kept in water at room temperature for about a year and then allowed to hatch. Table II. shows that this long period of rest produced no stimulation upon the race.

TABLE II.

Experiment.	Time, 1912.	Close-fertilized Eggs of Race *B* Kept in Water About a Year at Room Temperature.			Control.		
		Young Sisters whose Mother Developed from a Wet Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 2–17 Eve. 2–20	5	17	3.4	10	134	13.4
2	Eve. 2–17 Eve. 2–20	5	38	7.6	10	134	13.4
3	Eve. 2–17 Eve. 2–20	5	32	6.4	10	134	13.4
4	Eve. 2–17 Eve. 2–20	4	18	4.5	10	134	13.4
5	Eve. 2–17 Eve. 2–20	5	26	5.2	10	134	13.4
6	Eve. 2–17 Eve. 2–20	3	5	1.66	10	134	13.4
7	Eve. 2–19 Eve. 2–22	4	0	0	4	48	12
8	Eve. 2–19 Eve. 2–22	5	5	1	4	48	12
9	Eve. 2–19 Eve. 2–22	5	31	6.2	4	48	12
10	Eve. 2–19 Eve. 2–22	4	5	1.25	4	48	12
	Summary	45	177	3.93	14	182	13

Close-fertilized eggs of race *A* were dried and kept at room temperature for about eight months. Table III. shows the negative results obtained.

TEMPERATURE.

Of course, temperature has much to do with the state of activity of the particles of matter. In order to further test the hypothesis of inactivity some fertilized eggs of race *A* while still in water were kept at −70° C. for twenty-four hours. Other fertilized eggs were dried and kept in liquid air[1] at a temperature of about −191° C. for four days. Both lots of these eggs were hatched and the rate of reproduction of the developing females compared with that of the control. Tables IV. and V. show that no reinvigoration had taken place.

TABLE III.

Experiment.	Time, 1912.	Close-fertilized Eggs of Race *A* Dried 8 Months at Room Temperature.			Control.		
		Young Sisters whose Mother Developed from a Dried Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 2–21 Eve. 2–24	4	42	10.5	6	104	17.33
2	Eve. 2–24 Eve. 2–27	4	49	12.25	5	88	17.6
3	Eve. 2–25 Eve. 2–28	3	29	9.66	8	163	20.37
4	Eve. 2–25 Eve. 2–28	5	59	11.8	8	163	20.37
5	Eve. 2–25 Eve. 2–28	5	64	12.8	8	163	20.37
6	Eve. 2–26 Eve. 2–29	5	22	4.4	7	90	12.85
7	Eve. 2–26 Eve. 2–29	3	11	3.66	7	90	12.85
8	Eve. 2–26 Eve. 2–29	5	17	3.2	7	90	12.85
9	Eve. 2–26 Eve. 2–29	4	34	8.5	7	90	12.85
10	Eve. 2–26 Eve. 2–29	4	27	6.75	7	90	12.85
11	Eve. 2–27 Eve. 3–1	5	40	8	2	27	13.5
12	Eve. 2–27 Eve. 3–1	4	31	7.75	2	27	13.5
	Summary	51	425	8.33	28	472	16.85

Some dried fertilized eggs were placed at a high temperature of +100° C. for six hours. Table VI. shows that race *A* was not stimulated by this high temperature.

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[1] I am greatly indebted to Professor W. P. Bradley, of the department of chemistry of Wesleyan University, for his kindness in personally manufacturing and donating the numerous liters of liquid air which were used in these experiments.

Table IV.

Experiment.	Time, 1912.	Wet Close-fertilized Eggs of Race *A*, Kept at −70° C. for 24 Hrs.			Control.		
		Young Sisters whose Mother Developedfrom a Fertilized Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 2–14 Eve. 2–17	5	29	5.8	8	80	10
2	Eve. 2–14 Eve. 2–17	5	28	5.6	8	80	10
3	Eve. 2–14 Eve. 2–17	3	16	5.33	8	80	10
4	Eve. 2–14 Eve. 2–17	5	36	7.2	8	80	10
	Summary	18	109	6.05	8	80	10

Table V.

Experiment.	Time, 1912.	Dried Close-fertilized Eggs of Race *A*, Kept in Liquid Air at About −191° C. for Four Days.			Control.		
		Young Sisters Whose Mother Developed from a Dried Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 3–14 Eve. 3–17	3	29	9.66	5	92	18.4
2	Eve. 3–14 Eve. 3–17	2	21	10.5	5	92	18.4
3	Eve. 3–14 Eve. 3–17	4	44	11	5	92	18.4
4	Eve. 3–15 P. M. 3–18	2	11	5.5	6	73	12.16
5	Eve. 3–15 P. M. 3–18	2	7	3.5	6	73	12.16
6	Eve. 3–15 P. M. 3–18	1	7	7	6	73	12.16
7	A. M. 3–17 Eve. 3–19	3	6	2	4	32	8
8	A. M. 3–17 Eve. 3–19	3	9	3	4	32	8
9	A. M. 3–17 Eve. 3–19	4	23	5.6	4	32	8
10	A. M. 3–17 Eve. 3–19	2	4	2	4	32	8
11	A. M. 3–17 Eve. 3–19	3	13	4.25	4	32	8
	Summary	29	174	6	15	197	13.13

TABLE VI.

Experiment.	Time, 1912.	Dried Fertilized Eggs of Race *A*, Kept at + 100° C. for 6 Hrs.			Control.		
		Young Sisters whose Mother Developed from a Dried Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 3–4 Eve. 3–7	2	11	5.5	4	69	17.25
2	Eve. 3–4 Eve. 3–7	4	39	9.75	4	69	17.25
3	Eve. 3–4 Eve. 3–7	3	28	9.33	4	69	17.25
4	Eve. 3–4 Eve. 3–7	3	24	8	4	36	9
5	Eve. 3–4 Eve. 3–7	2	10	5	4	36	9
6	Eve. 3–4 Eve. 3–7	1	4	4	4	36	9
7	Eve. 3–6 Eve. 3–9	2	16	8	5	88	17.6
8	Eve. 3–6 Eve. 3–9	5	28	5.6	5	88	17.6
9	Eve. 3–6 Eve. 3–9	2	0	0	5	88	17.6
10	Eve. 3–8 Eve. 3–11	4	29	7.25	5	72	14.4
11	Eve. 3–8 Eve. 3–11	4	29	7.25	5	72	14.4
12	Eve. 3–8 Eve. 3–11	4	32	8	5	72	14.4
13	Eve. 3–8 Eve. 3–11	3	21	7	5	72	14.4
	Summary	39	271	6.94	18	265	14.72

TABLE VII.

Showing that there is no progressive decrease in the proportion of male-producing females in a long-continued parthenogenetic race. Male-producing females are designated ♂ ♀, female-producing females ♀ ♀.

Generations.	No. of Young Females Isolated.	No. of ♂ ♀.	No. of ♀ ♀.	Sterile Females.	Died.	Per Cent. of ♂ ♀.	Environment.
1–144	1434	181	1188	–	65	13.22	Watch glasses. Food was miscellaneous protozoa in horse manure solution, 7–28 days old.
510 and 527	116	37	71	3	5	31.89	Battery jars. Food was miscellaneous protozoa in horse manure solution, 7–14 days old.

CHEMICALS.

Professor Calkins was able to stimulate his weak races of paramœcia by diabasic potassium phosphate and also by the extraction of various glands and organs of certain mammals. Different percentages of diabasic potassium phosphate, extractions of the thyroid glands of the cat and sheep, of the thymus and adrenal glands, of the pancreas, spleen and liver, of the cat, Liebig's beef extract, and alcohol were added to the culture water in the watch glasses in which the rotifers were living. Only negative results were obtained.

DEATH OF THE PARTHENOGENETIC RACE *A*, AND ITS PROPORTION OF MALE-PRODUCING FEMALES.

Race *B* died out in March, 1911, in the 384th parthenogenetic generation, but race *A* was stronger and continued to reproduce parthenogenetically until June 12, 1912, when during the first hot weather the room temperature became higher than usual and this race died out in the 546th parthenogenetic generation. By its side was another unrelated race *C* in the 438th parthenogenetic generation. The latter race survived, since it was stronger, as is shown in Table I. Probably race *A* would have lived longer if the temperature had been kept lower, but eventually it would have died out, for at this time it took about four days for a generation of 4–6 young females to be produced while at the beginning of the experiments in October, 1908, it only took 1½–2 days.

Shull states: "A progressive decrease in the proportion of male-producers with long-continued parthenogenesis occurs in some lines of *Hydatina*, perhaps in all." This may be true in some races or lines of *Hydatina*, but it does not seem to be true for all races, as Shull is inclined to believe. Table VII. shows the proportion of male-producing females in the early and in the late history of this parthenogenetic race *A*. The environment probably was more or less different in the two periods from which these data was compiled so no especial emphasis can be laid upon the higher percentage of male-producing females that occurred near the end of this race. The influence of the environment in causing male-producing females to be produced has been presented in former papers. However, the point to be noted in this table is that this race *A*, which is near the point of dying out from general exhaustion after having lived through more than 500 parthenogenetic generations, is still capable of producing a high percentage of male-producing females.

Summary and Conclusion.

These results together with those of former papers show that three races of *Hydatina senta* kept under a constant environment gradually became weaker when allowed to reproduce parthenogenetically for several years.

Two races, *B* and *A*, have finally died out in the 384th and the 546th parthenogenetic generations respectively. The other race *C* has been discontinued in the 443d parthenogenetic generation but also showed a marked lowering of vitality.

2. A wide variation in the food media including the micro-organisms used as food and also the chemicals in solution did not reinvigorate the weak races. Extracts of beef, various glands of a sheep and cat, the diabasic salt potassium phosphate also were valueless as stimuli.

3. Close-fertilized eggs of the weak races subjected to periods of rest for a year in water and for eight months in the dried state, to a temperature of −70° C. in water and to temperatures of −191° C. and +100° C. in the dried state, 6–96 hrs., failed to produce any reinvigoration.

4. The final conclusions are: that parthenogenesis can continue for several hundred generations but results in the gradual weakening and final extinction of the race; variation in the environment including food, chemicals in the food media, and temperature, do not cause a reinvigoration in such weakened races.

5. Some races show no progressive decrease in the proportion of male-producing females with long-continued parthenogenesis.

Biological Laboratory, Wesleyan University,
Middletown, Conn., July 18, 1912.

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BIOLOGICAL BULLETIN

ECOLOGICAL SUCCESSION.[1]

V. ASPECTS OF PHYSIOLOGICAL CLASSIFICATION.

VICTOR E. SHELFORD.

CONTENTS.

I. Introduction 331
II. Basis and Method of Classification 335
  1. Basis 335
  2. Ecological Specificity and Specificity of Behavior 337
  3. Stratification or Vertical Aspects 339
  4. Habitat or Horizontal Aspects 343
    (*a*) Dynamic Relations of Local and Climatic Conditions 344
  5. Primary and Secondary Conditions and Communities 349
  6. Development of Ecological Classification 352
  7. Ecological Terminology 354
  8. Animal Communities in the Forest Border Region 357
III. General Discussion 359
IV. Summary 366
V. Acknowledgments and Bibliography 366

I. INTRODUCTION.

Every investigator appreciates the value of having several methods of organizing data on a given subject. Each new arrangement adds new light and often valuable conclusions.

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[1] The following errata appeared in the preceding paper, "Ecological Succession—IV.," Vol. XXIII., pp. 59 to 99 of this journal:

Page 66, Table I.: for *Lee* read *Dj.;* for *Psinida* read *Psinidia;* for *americanorum* read *americanus;* for *wyominganum* read *wyomingianum*; for *Cnemodophorus* read *Cnemidophorus.*

Page 67, for *arborens* read *arboreus* Say; for *Cyclisticus* read *Cylisticus;* for *Lyasopetalum* read *Lysiopetalum;* for *Spirobolis* read *Spirobolus;* for *promelia* read *pimelia;* for *Liobromum nigripalpi* read *Liobunum nigropalpi* Wood; for *hereculeanus* read *herculeanus;* for *Cerchus* read *Ceruchus.*

Page 68, for *melumboni* read *nelumbonis;* for *Ampulicidæ* read *Ceropalidæ;* for *Psithurus* read *Psithyrus;* for *anornis* read *anormis;* for *umbratis* read *umbratus;* opposite species 19 and 20, for *H* read *I.*

However in the general field of zoölogy, we have but one complete system of arranging data, namely the system of *taxonomy*. The recent attempts in ecology, while as yet scattered and incomplete, have been aimed in the general direction of the classification of organisms upon a *physiological basis with particular reference to relations to environment*. The relations of animals to environment were thought to be very orderly by many of the early naturalists but the lack of understanding of the environment led to difficulties. With the development of the idea of evolution, structural relations were sought, but aside from the "adaptations" of the large taxonomic groups to strata, etc., such attempts have, in the main, failed. The great importance which has been attached to the natural selection hypothesis has been largely responsible for the failure of naturalists to develop ecology along its proper physiological lines. This hypothesis, in the narrow sense in which it has been construed and applied, calls for life and death relations to environment, color and structural adaptations, and fixed physiological relations to environmental factors. Emphasis has been placed on the wrong phenomena; attention has been turned to pseudo-problems and some prejudice against all study of the environment and of the relations of the animal to the environment, has been developed.

On the plant side, present attempts at ecological classification date back at least to the publication of Warming's work in 1891, which followed closely after the general acceptance of the germ

Page 69, for *Byl* read *Gyl.;* for *fraudulentia* read *fraudulenta;* for *Pterostricus corecinus* read *Pterostichus coracinus;* for *Xylopodus saperaioides* read *Xylopinus saperdioides.* Table II., for *domocilorum* read *domicilorum;* for *Diaphoromera* read *Diapheromera.*

Page 70, for *Plectodera* read *Plectrodera;* for *Schistocera rubignosa* read *Schistocerca rubiginosa;* opposite species 11, for Locust read Pine.

Page 71, for *Tripleps* read *Triphleps;* for *guttivittata* read *guttivitta;* for *Scaphodius* read *Scaphoideus;* for *Lyngyphidæ* read *Linyphiidæ;* for *senitoria* read *senatoria;* for *Cryptophyllus perspecivius* read *Cyrtophyllus perspicillatus;* for *Symmirista* read *Symmerista;* for *anguissi* read *angusii.*

Page 72, for *obliturus* read *olitarius;* for *tubercolatus* read *tuberculatus.*

Page 82, for *Aglena* read *Agelena;* for *Dissostiera* read *Dissosteira;* for *Lyasopetalum* read *Lysiopetalum.*

Page 84, for *gracile* read *gracilis.*

Page 86, for *arborens* read *arboreus;* for *Tripleps* read *Triphleps.*

Page 97, for '*03*, Dahl, third reference, read '*08*.

Page 99, for *Woods-Jones*, read *Wood-Jones.*

plasm theory. This is, however, to be regarded as a renewal of interest turned aside at first by conflict with the church and later by the absorption of attention by the lines of work noted above, for the recognition of succession by Buffon preceded Darwin's "Origin of Speices" by one hundred and eighteen years (Cowles, '11) and occasional subsequent but early observations have strengthened the total evidence for this phenomenon. It is only a few years since we were regarding the environment as an ununderstandable hodge-podge. Now we must recognize that *environments are characterized by the most orderly of phenomena*, some of which were noted by early observers, and have often been verified and actually experimentally demonstrated during the last twenty years, by the plant ecologists. With this better understanding of the environments and the better knowledge of animal physiology, the relations of animals to environments considered physiologically appear particularly definite. Our knowledge is sufficient to enable one to indicate certain probabilities in this connection, which are based upon established principles.

In connection with the introduction of some of the principles of ecological classification and the logical necessities of such an attempt, the reader must not lose sight of the fact that there are, in practice, two points of view for investigation. One is that of evolution. The other that of physiology. One may make a physiological explanation of the behavior or structure of an organism and in no way explain its evolution, or on the other hand, he may contribute to the knowledge of the evolution of an organism without contributing to the knowledge of its physiology. This distinction is becoming less sharp with each year's progress in investigation, due merely to the adoption of physiological methods in the study of evolution and morphology. Again the reader must bear in mind the fact that regardless of widespread ideas to the contrary, *ecology* or *ethology* belongs primarily to the physiological point of view, and is therefore *outside of the range of criticism* from the point of view of *evolution* or the *current germ plasm doctrine.* Its frequent confusion with various branches of evolutionary speculation, such as mimicry, structural adaptation, etc., is one of the commonest errors of recent writers[1] and has been chiefly responsible for such prejudice as may possibly exist.

The definition of ecology, like that of any growing science is a thing to be modified as the science itself is modified, crystallized and limited. At present, *ecology is that branch of general physiology which deals with the organism as a whole, with its general life processes, as distinguished from the more special physiology of organs* (Semper, '81). With these limitations upon the term physiology, what may be termed *physiological life histories* (Ganong, '07) covers much of the field. Under this head fall matters of *rate* of *metabolism*, *latency* of eggs, *time* and *condition* of *reproduction*, necessary conditions for existence and especially *behavior in relation to the condition of existence.* Reactions of the animal maintain it in its normal environment; reactions are dependent upon rate of metabolism (Allee, '12, and citations) which may be modified by external conditions. Behavior reactions throughout the life-cycle are a good index of physiological life-history characters. If we knew the physiological life histories of a majority of animals most other ecological problems would be easy of solution. The chief difficulty in ecological work is our lack of knowledge of physiological life histories. On this account the relative importance of the different aspects of investigation given later in this paper is based upon present expediency.

Physiological life histories may, with elaborate facilities, be worked out in a laboratory. *Ecology* however considers physiological life histories *primarily in nature* and for this reason as has already been stated the central problem of ecology is the *mores*[2] problem or the problem of physiological life histories in relation to natural environments, the dominant facts in which are facts of *behavior*, *habitat-preference*, *community* of habitat preferences and *laws governing* the *relations* of *organisms* in *communities*. The last is not a part of physiological life histories, the *mores* conception being broader than that of physiological life histories. An ecological classification is a classification upon a physiological basis, but since structure and physiology are inseparable, we must also note the relations of structure to ecology and to ecological classification.

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[1] See *Trans. Am. Micro. Soc.*, Vol. XXX., p. 217.

[2] *Mores* (Latin, singular *mos*) "behavior," "habits," "customs"; admissible here because behavior is a good index of physiological conditions and constitutes the dominant phenomenon of a physiological life history in the sense proposed. We have used the term just as *form* and *forms* are used in biology; in one sense to apply to the general ecological *attributes* of motile organisms; in another sense to *animals* or *groups of animals* possessing particular ecological attributes. When applied in the latter sense to single animals or a single group of animals the plural is used in a singular construction. This seems preferable to using the singular form *mos* which has a *different* meaning and introduces a second word. The organism is viewed as a complex of activities and processes and is therefore a plural conception.

II. Basis and Method of Classification.

1. *Basis*.

The ecological distribution of *mores* is the resultant of the behavior reactions of the animals constituting them, to variations of environmental conditions, encountered in their movements in space. According to the law of toleration (Shelford, '11^3), the distribution of species or mores is limited by the variation of a factor or factors beyond the limit of toleration of the species or *mores* in question. The statement of the law was based upon field and experimental observations on the tiger beetles, which clearly supported this view. We noted that where the conditions were nearest optimum, the number of larvæ was greatest and where least favorable the number was smallest. It appears, from field study, that the number of individuals varies directly with the degree of deviation from the conditions most favorable. The distribution of each species or *mores* is probably representable by the ideal curve (see Fig. 9, *p.* 351) when viewed with reference to all environmental factors. As we pass in *different directions* from the point of maximum number of individuals, *different* factors or combinations of factors are seen to fluctuate. The area of maximum numbers is the area of overlapping of optima of the various factors. This is called the *ecological optimum* (Schimper, '03). The ecological optima of many mores are similar, a fact well known to naturalists. The fact is most evident where changes in conditions are most abrupt. Ecological classification places together groups of *mores* with similar ecological optima.

Ecological classification, whether of groups of individuals belonging to a single species, or of communities composed of all the animals of a locality without regard to their taxonomic relations, is based upon differences and similarities of *mores* or general physiological characters. These differences in *mores* are measured (*a*) by the direct study of the organism, and (*b*) by the study of the environment of the organism or the *mould into which the organism fits*. As a background for our point of view, we have, under the first heading, various experimental studies of adjustment of the behavior of organisms to surrounding conditions, especially studies of the modifiability of behavior, which has been definitely related to conditions which the organisms concerned encounter in their normal life in nature; under the second studies of the selection of habitat by organisms. My own studies (Shelford, '11[3]) from this point of view are at present very incomplete and serve to illustrate the methods only. Different tiger beetle species select different soils and as the females lay only one season, their first attempts at laying no doubt are the result of innate behavior (Shelford, '07). The work of many investigators (Wheeler, '10, *et al.*) confirms the general view that animals select their habitats upon the basis of characters more or less innate. The work of naturalists is important though it is defective mainly in that one often has difficulty in determining what habitat is meant.

A type of investigation which combines experimental and naturalistic consideration of the organism with analysis of the environment has been carried on by Allee ('12). He found that the rheotaxis of isopods of the same species occurring in both ponds and streams, is different in the two situations. He was able to change the pond *mores* to the stream *mores* by keeping pond isopods in stream conditions and vice versa. The agreement of the behavior of the animals of a habitat will be shown by a study of the behavior of the swift brook community now being conducted. There is a marked agreement of the animals of this community in their reactions to the factors encountered in the stream. This agreement is due (*a*) to the *selection of the habitat through innate behavior*, and (*b*) to the *adjustment of behavior to the conditions* through the effects of physical factors and through formation of habits and associations.

The study of the mould into which the organism fits becomes a *legitimate method as soon as the selection of habitat and the adjustment of behavior and physiological makeup, to the environment are shown to be general facts.* The study of the environment must be accompanied by studies of the *effects* of the *various factors* upon the *organisms* concerned. This is necessary if important factors are to be emphasized (Shelford, '12[1], p. 90; Shelford and Allee, '12).

2. *Ecological Specificity and Specificity of Behavior.*

We have stated that ecological classification is dependent upon *similarities* and *differences* in *mores* (physiological life histories, behavior, modes of life). We have noted also (Shelford, '12[1]) that there is *similarity* of mores within the same animal community and that the limits of animal communities are based upon similarities and differences of *mores.* Several questions at once arise. Is the same environment ever the same to different species? How much and what kind of similarity is to be expected? It is possible for two very different species to live under practically identical conditions, but probably this is rarely true in the same community, such cases usually being *separated geographically.* If the organs for the reception of stimuli, on the bodies of two animals living side by side, are differently placed, there must be obvious differences in reception of mechanical stimuli, light, etc. Indeed different species living under similar conditions may be sufficiently different physiologically to be differently affected by the same single stimulus, but ecologically, classification is based upon the *complete physiological life history,* mode of life, and behavior, so that differences in the effect of *single stimuli* cannot be too much emphasized.

It is quite clear to every naturalist, that within a given area, nearly every race or species possesses certain special peculiarities of structure and also of behavior, physiology, and mode of life. There is a large amount of specificity in the behavior of a species and as a rule, students of behavior have been unduly impressed by it. For example fishes (Shelford and Allee, '12) show a general community or similarity of reaction to such factors as carbon dioxide and other differences in water. The fishes turn back when they encounter increased carbon dioxide or other differences in dissolved content in the water, doing so without regard to the specific peculiarities of their behavior, such as methods of moving their tails, mouths or opercles. By way of further illustration, we note that, according to the accounts of naturalists, there are striking resemblances between the behavior of some of the antelopes of the savannas of Africa and certain of the savanna kangaroos of Australia. In other words certain kangaroos are ecologically similar to some antelopes. As has already been stated, the zoölogist is usually unduly impressed with specificities such as mode of movement of limbs, body, etc. Now if my reader pictures an *African antelope running gracefully from a pack of Cape hunting dogs* (Selous, pp. 119–123) *and an old-man-kangaroo leaping from a pack of dingoes* (Ward, '07, pp. 41, 243) *noting mainly the specific peculiarities of the movement of limbs and body of the pursued in each case*, he will be dwelling upon *specificities of little ecological significance and missing the point of view of the ecologist altogether.* These specificities of behavior are matters of little ecological significance; it matters not if one animal progresses by sommersaults so long as the two are in *agreement* in the matter of *reactions* to *physical factors* as indicated by the manner of *spending* the day,[1] *avoidance of forests, swamps, cold mountain tops, etc.*, entirely *available* to them, and in the mode of meeting enemies as indicated by the reaction to the approaching enemy—a relation to other animals of the community. As a further example, the specific method of avoiding stimuli shown by *Paramœcium* is not a matter of any considerable ecological importance. The chief argument against ecological classification is based upon specificity of behavior. With all the marked specificities there can be no similarities! Let us apply this logic to a few particular cases. Since there are specific differences in the behavior of different fish species, different fish species do not turn back from carbon dioxide in a similar way and are not similarly affected by it! Since there are species and no two species of a genus are alike there can be no genera; since there are genera each with definite characters, there can be no families, etc. *Specificity* of *behavior* comes in ecological classification or other ecological consideration as a matter of tertiary or even quaternary significance, *even when details are being considered.* This applies to particular *mores* (ecological species) as well as to groupings of higher order. Ecological specificities are primarily *differences in physiological life histories manifested mainly by (a) details of time and place of reproduction and degree of latency in reproductive structures, and (b) by quantitative differences in reactions to the same intensity of the same environic factors.* Because of lack of knowledge of life histories, the latter will doubtless be most useful in practice. It is also the best test of animals temporarily invading a community to which they do not primarily belong. Such animals should be in partial agreement with the communities which they have entered even though their residence there be temporary.

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[1] Lydekker, III., 243; Vol. II., p. 322; Riverside, N. H., Vol. V., pp. 36–37.

3. *Stratification or Vertical Aspects.*

(*a*) *Adaptation.*—In the preceding paper we divided the animal communities into strata. Persons not familiar with ecology appear to think that structural adaptations are an important part of the consideration of modern ecology. In the first place, ecologists are skeptical of the significance of many if not of the majority of so called structural adaptations. In general, structural adaptations appear not to be correlated with the phenomena with which the modern ecologists are concerned. For example we note (Shelford, '07, '12[1]) four species of tiger beetles arranged in the horizontal series of conditions which we find at the south end of Lake Michigan. A careful study of the adults and the larvæ of these species fails to show any structural characters which are correlated with the conditions in which the species live. All have the same type of mandibles, the same kind of feet, and the same kind of ovipositor. There are no structural characters by which they can be located in their environments. The adults are structurally adapted to making holes in the ground with their ovipositors and thus depositing their eggs. The larvæ are adapted to a life in the ground. These are structural adaptations to *stratum.* All terrestrial tiger beetle species are somewhat in agreement as to adaptations. Other adaptations among the tiger beetles are adaptations for walking on leaves of plants (*Odontochila*, Bates, p. 169), for creeping on the trunks of trees

(*Derocrania*, Horn, '99, pp. 228–230), for depositing eggs in twigs (R. Shelford, '07). Apparently several of these types may occur, one above the other, in one locality, or at least at different levels in adjoining localities (R. Shelford, '02, pp. 233–234).

Among the Orthoptera we find forms adapted to burrow beneath the soil, others which live at the surface of the soil having ovipositors adapted to deposit eggs in soil, feet adapted to life on soil, etc. Those that live on the shrubs are adapted to walk on vegetation, and to deposit eggs in plant tissues (Morse, '04). Motile aquatic animals are adapted to burrow into the bottom, to cling onto the bottom, to cling on the vegetation or zoöphytes, or to swim about. Feeding adaptations in the fishes are to feed in the open water, in the vegetation, or on the bottom, each of which is a relation to stratum or matters comparable to strata.

Mammals are adapted to aquatic, subterranean, cursorial life. By way of further example, let us take the pocket gophers which occur (Merriam, 95) in the subterranean stratum of all the great steppes, deserts, and dry and moist forests of the United States and Mexico, apparently from the most arid deserts to the moistest tropical and subtropical forests. Adaptation limits their relations only to stratum. Again cursorial mammals, with all possible numbers of toes, are found in all of the climates of the world, in the forests, steppes, and deserts, arctic and tropic, all being adapted to the ground stratum. The arboreal types are likewise widely distributed. Arboreal monkeys, for example, occur from the snow covered pines of the Himalayas (Heilprin, '87) to the moist unchanging forests of the Amazon, all being adapted to the tree stratum. In many taxonomic groups, such as families and even genera, we find structural characters which seem fitted to various levels of habitats, but which do not limit the animal to any particular ecological *types* of plants growing in any particular set of physical conditions. Neither do they, in many cases, seriously limit its mode of activity. Adaptations to stratum appear in many cases to be quite elementary, occurring within genera, while other adaptations such as those for food getting, belong to larger taxonomic groups such as orders and suborders. Those of higher order are however never of primary ecological significance. Take the piercing mouth parts of the Hemiptera. This group occurs in every type of habitat from the marine pelagic to the ectoparasitic, and from tundra and desert to rain forest. All the chief principles of animal ecology could probably be illustrated by the Hemiptera, the sucking beak coming in only as a factor modifying the details, when we compare Hemiptera with Coleoptera which have biting mouth parts and similar habitat relations.

Such doubtful protective devices, as protective coloration, mimicry, aggressive coloration, etc., cannot be counted as any significant part of ecology until they are first established in fact and are shown to have some regular relation to reactions to environic factors or at least to activity. All of the chief typical cases that come under the head of protective coloration, mimicry, etc., are much shattered by such facts as are presented by Selous ('08, Ch. I. and II.).

(*b*) *Over-adaptation.*—If some animals are adapted, which implies that they are adjusted to a particular mode of life in their particular situation, in a way which is essential to a successful life in that situation, then other animals are over adapted (Coulter, '09, p. 62). Take an animal from the insect group, the dragon fly nymph, which has the labium modified as a prehensile organ and the maxillæ as additional mandibles. The posterior portion of the intestine is developed into a muscular cavity containing gills and serving as an hydraulic organ of locomotion. What is the advantage of all this specialization? The nymph appears to succeed no better than many other types with which it is in competition. It even appears clumsy and unadapted in many ways and is to the same degree *over-adapted.*

When we consider adaptations in relation to communities of organisms and to physical environments, with taxonomy thrown into the background, their significance loses force. Adaptation is adaptation primarily, when viewed from the standpoint of the structural type of the group to which the adapted organism belongs; it is an index of *taxonomic differentiation* rather than of *ecological relations.* The more important structural adaptations appear to be adaptations to *strata,* or matters of *specificity.* They have about the same significance among animals as the separation of plants into herbs, shrubs, and trees, has among plants. They merely represent, in the main, the different taxonomic groups primarily capable of occupying different strata or the like. Here and there an aberrant member has become adjusted in size or growth form to another stratum. Climatic grassland formations (Shelford, '11[3]) may perhaps be characterized by the absence of arboreally adapted animals but even here we may find exceptions, for *Didelphys azore* lives in grassland (Hudson, '03) but is unmistakably "adapted" to an arboreal life. Again in the rain forest of New Guinea, we find a tree kangaroo which is poorly adapted to arboreal life (Wallace, '69, p. 386).

When one brings together all the motile animals of a given stratum, in a forest or other type of habitat, and examines them with a view to classifying and generalizing concerning them, from the point of view of structure, he finds himself confronted with a hodge-podge of the so called "primitive," "adapted" and "unadapted," without the possibility of making any generalizations concerning them or of classifying them into structural ecological groups.

Among motile animals, structure must be considered in connection with activities, reactions, and general physiology as a limiting factor to be taken into account in many cases. It is often important in considering the ecological equivalence (Shelford, '11[3]) of similar communities or of the animals of the same community. Ecologically there is rarely reason for considering the structure of motile animals separate from activity. Accordingly it seems best to reject separate consideration of adaptation and to treat all questions of the structure of motile animals as *structures playing a rôle in the physiology and activity of the organism.* This includes all the important aspects without raising the question of adaptation or of the origin and genetic significance of such structures concerned. Even the relations of the animals to the strata are to be considered as *primarily physiological relations* to differences in *physical conditions*, such as were brought to attention in the preceding paper. Many animals invade two or more strata and are usually to be classed primarily in the stratum in which they breed. On account of this invasion of several strata, there is much overlapping of adjoining strata corresponding to overlapping of single characters in taxonomy.

4. *Habitat or Horizontal Aspects.*

Ecological classification is not only vertical but also horizontal. Some of the roughly horizontal aspects of classification, such as division into aquatic (marine and fresh water) and terrestrial (forest, prairie, etc.) are major aspects of classification long in use. Animals invade several of the minor recognizable *horizontal conditions less often than several of the vertical.* Still such overlappings are common, and as has already been noted, since overlapping of single characters is common in taxonomy, it cannot be urged as argument against ecological classification.

(*a*) *Large and Small Divisions.*—Considering terrestrial communities, the largest are those that occupy areas of relatively uniform climate (climatic, Schimper, '03: major; Adams, '08; Shelford, '11[3]). The smaller those of similar soil (including water) (edaphic, Schimper, '03), of similar degrees of exposure to wind, sun, etc. due to the topography (local or secondary, Adams, '08; Shelford, '11[3]). Each area when considered as an environment includes the vegetation, which is usually clearly different in growth-form in each area occupied by a different animal community.

Both local and climatic communities may be subdivided into still smaller but easily recognizable subdivisions. Their number is closely related to severity of climate, particularly with reference to moisture, and is greatest in the desert and smallest in the rain forest. For example, in dealing with forest development in the preceding paper (Shelford, '12[1]) we noted that in the earlier stages, conditions were dominated by the presence of *bare sand* open forest communities. The later stages were closed forest with the soil all covered and the character of the forest and of the animal communities quite *independent* of *soil* and *dependent* upon *climate.* Still each of these sets of conditions was divisible into several subdivisions, the sand dominated habitat into the cottonwood, the pine, and the black-oak habitats, each with a different rate of evaporation and each with different soil conditions along with recognizable differences in communities. The closed forest was likewise divisible into three habitats and three corresponding communities, the blackoak-whiteoak-redoak, the redoak-hickory, and the beech-maple.

(*b*) *Dynamic Relation of Local and Climatic Conditions.*—There are many local conditions in each climatic area. The relation of local conditions to the climatic or major is closely bound up with the principle of succession. In the preceding papers we have noted that succession may be due chiefly to physiographic changes, or to the fact that the organisms of a given stage affect conditions in such a way as to make their own continued existence impossible, and prepare the way for others. While in *particular cases*, *physiographic* conditions may dominate, in others *biological* conditions dominate. Both are probably always detectable factors. (Cowles, '11; Adams, '01, '08.)

Under the head of physiographic causes of succession come such processes as the uplifting and degradation of land, erosion, deposition, etc. Along a coast, the processes (Gilbert, '85; Gulliver, '99; Salisbury, '07) are causes of changes in physical conditions, material for abode, etc., and result in ecological succession. Such geological processes are treated in textbooks on geology and physiography and may be only outlined here.

When a body of land is uplifted or the level of the water into which it is drained is lowered, streams begin to work their way into the new land mass and cut deep valleys, with marked differences in both vegetation, physical conditions, and animal communities (Shelford, '11). The formation of numerous tributaries (see diagrams by Salisbury, Adams, '01) isolates portions of the upland in the form of hills. These hills are broken up into smaller hills by the smaller tributaries, and the resulting hills into still smaller, until the upland is all removed and the country reduced to a generally rolling topography with very little relief and known as a peneplain (Adams, '01; Salisbury, '08; Chamberlain and Salisbury, '06). The process of peneplanation then tends to fill all low lakes and ponds and to drain all high ones. It works over all of the materials of the upland and deposits them over much of the resulting surface, which tends to make the surface materials of a uniform nature. The processes involved go on in definite directions during longer or shorter periods and produce smaller and larger differences in conditions, due to topography, but all of these point toward a common end, the peneplain. Peneplains may be local being referable to some

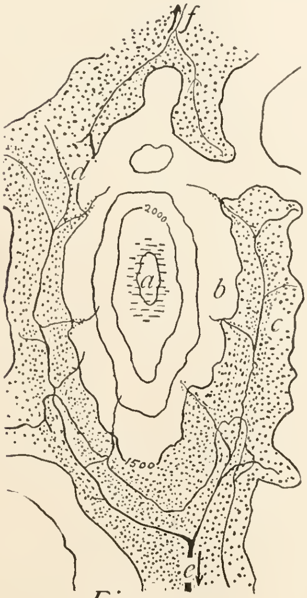

Fig. 1

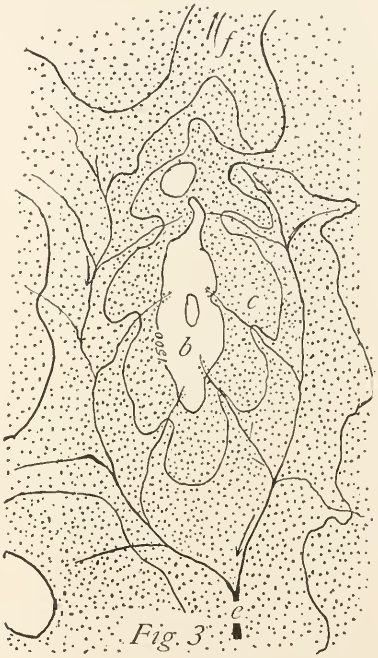

Fig. 3

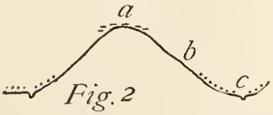

Fig. 2

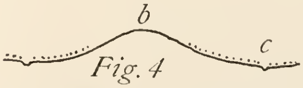

Fig. 4

FIGS. 1 and 2. Showing a mountain in east Tennessee [Briceville Folio, U. S. Geological Survey, Latitude 36° 30′ N. Longitude, 84° 5′ W. It is taken from the topographic map with some of the contour lines omitted. The contour interval is 250 ft. (80 meters)]. In Fig. 1 the area (*a*) indicated by the dashes is at the top of the mountain and represents the area covered by conifers. The blank area (*b*) represents the area of the mountain side covered with the oak and hickory forest the habitat of *Cicindela sexguttata*. The stippled area (*c*) represents the beech and maple in the valley. Fig. 2. Cross section of the same.

FIGS. 3 and 4. Showing the same mountain represented in Figs. 1 and 2, but in an hypothetical later stage, based on the supposition that the valley at the point (*e*) is at a peneplain level. In Fig. 3, stream (*e*) has cut through at the point (*d*) of Fig. 1 and captured the head-waters of the stream (*f*). The entire mountain has been sufficiently lowered to cause the conifer area to disappear entirely. The oak-hickory area is greatly reduced and the beech-maple is greatly increased, Fig. 4 is a cross section of the same (see Adams, '01).

large inland body of water like Lake Michigan (Atwood and Goldthwait, '08) or they may be extensive. In both cases the processes proceed in a definite direction.

In eastern North America, the topography of the Appalachian region is in the main features of importance in this connection, an erosion topography. In eastern Tennessee I found [as described by Cowles (unpublished)] that the tops of the mountains were frequently covered with conifers, the sides with oak and hickory, and the bases with beech and maple. In Figs. 1 and 2, the area marked with dashes is conifers, the blank area is oak and hickory, and the stippled area beech and maple. Each was occupied by different animal communities. The beech and maple are at a level at which the whole area will be when the mountain is reduced to a peneplain level (unpublished conclusion of Cowles). Hills with isolated patches of conifers at the top are numerous throughout the Appalachians. Hills covered with oak and hickory without the conifers, and surrounded by beech and maple and the other mesophytic trees that grow with these, were doubtless very common in the foothills of the Appalachians under primeval conditions.

Turning to Figs. 1 and 2 we note that as the height of the mountain is reduced the low beech area becomes larger, at the expense of the oak and hickory habitat, and the oak and hickory habitat in turn adds to itself at the expense of the conifers. In Figs. 3 and 4 is shown an hypothetical stage in which the stream has cut through the upland and captured the headwaters of the stream at point *d*. This has completely isolated the oak-hickory. It is completely surrounded by the beech and maple. The conifer community has disappeared. Peneplainations have taken place completely. For example, remains of ancient peneplains are recognized in the Appalachian region, each corresponding to a relative lowering of the level of the sea. The first was complete. The second was sufficient in extent to cause the isolation of numerous uplands and groups of mountains. The erosion processes now in progress have still further dissected the land into uplands of all possible heights, between the upper and lower limits. Each peneplanation was accompanied by changes in physical conditions, in vegetation and by ecological succession of animal communities (Adams, 01).

Succession proceeds until conditions are such as to be favorable to organisms which are immune to their own effects upon their own environment. Ecological succession proceeds from all *mores* types toward the physiological types of organisms which are adjusted to climatic conditions of the area. On the basis of good evidence, which cannot be reviewed here, but which is to be found in the writings of Cowles ('01[1], '01[2]; Clements, '05; Gleason, '08, '11; Adams, '08[1], '08[2]; Whitford, '06) and others, botanists have reached the conclusion that the vegetation and therefore the chief animal habitats of the local conditions are converging toward a climatic type immune to its *own excretory products*. This has been called the climatic climax. The diagram (Fig. 5) shows some of the striking stages in convergence in northern Indiana. See also diagrams by Gleason ('08, p. 78, and '10, p. 133).

SAND RIDGE
V, A, 1—Cottonwood
V, A, 2—Gray pine
V, A, 3— Black oak
V, B, 1 { Black oak, White oak
V, B, 2 { Red oak, Hickory

CLAY BLUFF
Aspen, Cottonwood } near IV, B.
Hop-Hornbeam, White oak } V, B, 1
Red oak, Hickory } V, B, 2

[*Rana sylvatica Association*]
BEECH AND SUGAR MAPLE, V, B, 3
[*Rana sylvatica* Association]
Tulip
Basswood
White elm and white ash
Hickory, Red oak } V, B, 2
Bur oak

IV, A, 2 { Swamp whiteoak, Buttonbush
II, C, 2 { Cat-tail and Bulrush, Water lily and Water Mill-foil
II, C, 1—Chara
POND

Bass-wood
Hawthorn
Slippery elm and white elm
River maple
Black willow—IV, A, 1
FLOOD PLAIN

FIG. 5. Showing convergence of four types of habitats in northern Indiana to the beech and maple forest. Prepared with the assistance and from the writings of Dr. H. C. Cowles. Read from the extremes toward the center. The figures and letters standing outside the names of the trees refer to the communities similarly numbered in the list on pp. 358 and indicates the plants with which they are associated. The absence of numbers in connection with a number of the plants is due to the incompleteness of the lists in question.

The principle of convergence, while not generally established is believed to be of wide application. It has been suggested, in connection with the tropical forest about Mount Marivales, in the Lamoa Forest Reserve (province of Bataan, island of Luzon, P. I.; Whitford, '06) where the rainfall is great. Here the difference between the vegetation of a ravine and the adjoining upland is less than in the temperate regions. The principles are, however, the same as those established in the temperate regions of America, Fig. 6.

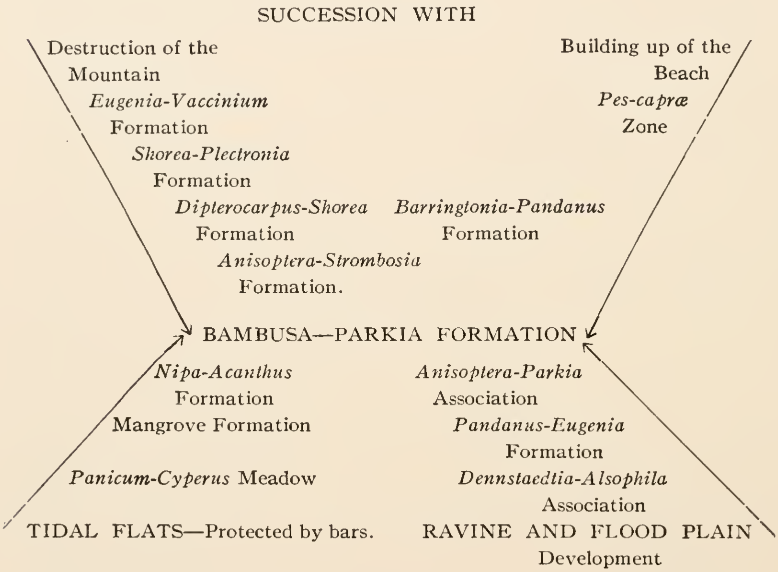
SUCCESSION WITH
Destruction of the Mountain
Eugenia-Vaccinium Formation
Shorea-Plectronia Formation
Dipterocarpus-Shorea Formation
Anisoptera-Strombosia Formation.
Building up of the Beach
Pes-capræ Zone
Barringtonia-Pandanus Formation
BAMBUSA—PARKIA FORMATION
Nipa-Acanthus Formation
Mangrove Formation
Panicum-Cyperus Meadow
TIDAL FLATS—Protected by bars.
Anisoptera-Parkia Association
Pandanus-Eugenia Formation
Dennstaedtia-Alsophila Association
RAVINE AND FLOOD PLAIN Development

FIG. 6. Showing succession and convergence in a tropical climate. Read from the extremities toward the center. Beneath the word "mountain" is shown the succession of formations with peneplanation; beneath "beach" with the building up of the beach (when it has been built up above high tide, the succession proceeds without physiographic change); above "ravine" and "flood-plain," and above "tidal flats," succession with the development and final destruction of these features. Compiled from Whitford's account, with the assistance of Dr. H. C. Cowles.

Whitford's statement regarding the climax forest of this tropical area is as follows (p. 679): "The physiography of the entire mountain is very unstable and therefore the vegetation is temporary. With its (the mountain's) destruction, it will pass to a more and more permanent condition and the vegetative types will be altered with the change in the topography; the climate will then also be different, and will more nearly approximate that at present prevalent at the base; therefore the erosive topography at old age will have a vegetative condition not unlike the *Bambusa-Parkia* formation. Just before the death of such a topography, the whole country will be brought nearly to base level (peneplain) with the ground water near the surface. The vegetative conditions will not be unlike that of a delta region, of which there are many fine examples in the Philippines. . . . Of course the above are only theoretical considerations, yet these erosive stages are approximated in different parts of the island, so that when logically united, the genetic relations of the different vegetative formations can be made clear."

Physiographic processes and the processes of plant succession go on at varying rates under different conditions. Where the land stands low, is of easily eroded material and in a rainy district, a peneplain may develop over a considerable area in a few thousand or even hundreds of years. The same process would require infinitely more time in an arid region. With favorable soil conditions, a shallow pond will pass through all the stages of succession and into prairie or early forest in two or three decades. In moist climates, young forest springs up in a comparable period. On the other hand, the peneplanation of large areas, climatic changes, etc., require longer periods but *follow* the *same general laws as do those changes which take place quickly*, and ecological succession is similar in principle no matter whether the changes are slow or rapid. *Animal habitats and animal communities are orthogenetic and converging* (Adams, '08).

5. *Primary and Secondary Conditions and Communities.*

Every system of agriculture is, ecologically speaking, the holding back of all natural changes due to the effect of organisms. It consists in preventing the operations of all the biological laws, by which changes in the character of the habitat are brought about. If the organization of ecological materials is to be brought about in correlation with natural laws, then *agricultural* communities are essentially *useless* subjects for study.

Plant ecologists have recognized a division into primary and secondary communities (Warming, '09). The *primary com-*

munities are those which are normal to a given geographical area, while the *secondary communities* are those produced by cultivation or other activities of man, and are maintained only by cultivation, reverting to the primary types as soon as the cultivation ceases. They therefore represent ecological experiments with the essential records usually entirely lacking, and with the habits of the species constituting them undetermined, so that until the primary communities have been thoroughly investigated, the value of such experiments cannot be ascertained. Failure to recognize the division into primary and secondary is responsible for much of the confusion in the matter of environmental relations of animals in general, but more especially of land animals.

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rty dbhty fbhtygfh dbgfty
dftr fgythrtg bfgtyr hufgk
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bcvfdtxnzxdfcrtfbhtgydbfgg
vb fnhtbgfy fgyr bfgvdftrt
bfgtrybdbfghty vbft dgtrxd
vbgf tg fbgtyhyuijghb f nb
dfcrgtryhfy vbgfhty dbgfty
cbvfghtygdtfrnvkjgh fbgtb
cvfdgfbt nhkzxdscxgczkzkvg
bfgtyhr dftrgtybvhfgiingvb
dfcrg cgbfhty du bkbkbbgfh

FIG. 7.

Showing the arrangement of plants and animals on a plot of ground under primeval conditions, each letter representing several closely associated plants and animals.

edge rftfgyrhtubfhgtybgft
fecdftrybthuginf dvgfhytb
cb a a a a a a a a a a bf
ed a a a a a a a a a a ed
fg a a a a a a a a a a fg
dc a a a a a a a a a a dc
eb a a a a a a a a a a dg
fd a a a a a a a a a a fd
dc a a a a a a a a a a dc
fe a a a a a a a a a a fe
eg a a a a a a a a a a eg
fc a a a a a a a a a a fc
cgnvdftrgghhfgdbfbdgtrycg
gfzcvdg cvbdg fvgty ggd b

FIG. 8.

Showing the arrangement under agricultural conditions. Some of the original groups are to be found along the fence rows.

When civilized man first enters a new territory, he destroys or attempts to destroy, large game which threatens him and his domestic animals. He then destroys the natural vegetation and incidentally many other animals, by clearing the timber and burning all woody débris, and plowing and putting out plants which are entirely new to the region. Under primeval conditions plants are arranged irregularly as roughly indicated by the letters in Fig. 7. After the land is put to agricultural uses, they are arranged as in Fig. 8. Here the plants are all of one kind and are arranged in rows. A grove of the original vegetation is sometimes left, and some of the original plants remain near the fences, etc.

The fence rows and road sides are usually inhabited in the forested districts, and often also in the prairie by forest margin animals. The weedy, shrubby roadside and fence row is duplicated in the abandoned fields which are common on the poorer soils. In a forested area, some forest animals live after the clearing process is finished, in fence corners, under stones, etc. The distribution of a given species with respect to *conditions* is representable as the solid of an ideal curve, Fig. 9, the modal portion of the curve being the optimum conditions for the species in the forest. When the forest is removed, if we assume that the species can still live for a time, we find it in several different situations, which represent the outskirts of the range of toleration. Such places are protected ravines with bushes, fence corners, and partially cleared woodland. None of the situations lies within

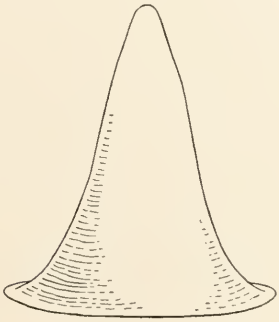

Fig. 9

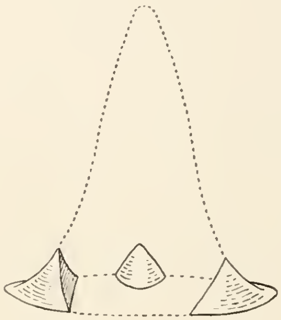

Fig. 10

FIG. 9. The solid of an ideal curve, representing the distribution of numbers of individuals of a *mores* with respect to degrees of variation of environic factors in space but without reference to distance or area covered by the degrees of variation. The central or modal portion is the area of ecological optimum.

FIG. 10. Showing the distribution of the same *mores*, after the natural vegetation has been supplanted by agricultural plants and the *mores* has been left in the fence rows, roadsides, ravines,—situation which represented the outskirts of its possible range under primeval conditions.

the area of optimum. A study of the ecological distribution of the animals thus located gives no correct idea of the ecological optimum, but makes the relations of the species to conditions seem *particularly variable*. From the point of view of ecological generalization, data on distribution under agricultural conditions are of questionable value.

6. *Development of Ecological Classification.*

The separation of animals into marine, fresh-water, and terrestrial has long been practiced; such a classification has about the same significance as the division of animals into vertebrates and invertebrates. Some other divisions have been recognized but usually rather loosely and little could probably be added by the study of literature which exists. A few of the recent attempts at ecological classification deserve mention. Three principal classifications by zoölogists apparently not in close touch with progress on the plant side must be noted. Morse ('08) divided the Orthoptera into *geophiles* and *phytophyles* representing certain "structural adaptations" (to strata). Both of these main groups he further divided into *xerophiles* and *hygrophiles*, and each of these in turn into *campestrian* and *sylvan*, etc. The classification is dependent primarily upon strata. The factors involved are unanalyzed and the scheme fails to distinguish the differences in physical conditions, which will probably come to be the basis for all ecological classification. For example, shrub-inhabiting species are not divided into those inhabiting *thickets exposed to sun and atmosphere in the open* and those inhabiting thickets or shrubbery in the *shaded forest*. Two groups wholly unlike in their relations to physical factors are thus put together. Shull ('11) points out the failure of the classification in practice.

Hancock ('11) follows a plan similar to that of Morse but makes it much more complete, on the whole, contributing much to the knowledge of ecological distribution of the Orthoptera. He fails, however, to separate primary (primeval) conditions from secondary (agricultural and human) conditions, and like Morse, has made divisions primarily upon the basis of strata or levels, with only partial consideration of physical factors or physical conditions. A reviewer (Pearse, '12) of Hancock's book (Shelford, '12) appears to have thought that this classification followed that of plant ecologists, but the main difficulty with this classification and that of Morse, is that they do not follow the plant ecologists' classification in any of its essential features. Both made the primary division vertical, though frequent invasion of several strata by the same animals makes the application of such a system to entire communities a practical impossibility. Such invasions of the recognizable horizontal divisions are far less frequent. Dahl ('08), in a list of places from which animals may be collected, gives a good classification of animal habitats and partially separates the primary and the secondary conditions. His general outline possesses many points of merit, and is probably the best published list. The author's comprehensive knowledge of the subject is clearly indicated throughout. It is not, however, arranged with particular reference to physical factors, and differs from the attempts of the American ecologists in that it is not based upon the laws of evolution or succession of environments.

The advantages of following natural laws, in ecological classification, should not need elaboration, for such has been the chief guide of all systems of classification of scientific data, at least since the time of Darwin. Elements of progress in ecological classification, following natural laws governing animal and plant habitats, have come chiefly from American ecologists, and largely, no doubt, because in the newer country there has been more opportunity to study the laws of succession. In Europe such laws can more rarely be studied, because of the more intensive efforts of man to prevent the operation of such laws.

In this country, Cowles was the first to make use of the laws governing plant habitats, in the classification of plant communities. Warming who contributed to the field before Cowles, took the condition of the plant as his main guide, but clearly recognized the laws governing habitats as of importance. Clements has also added much on the plant side.

Adams ('08) and Gleason ('08) arranged the animal communities of Isle Royale, Lake Superior, according to laws of succession, and gave us important elements of progress. Livingstone, Brown, Transeau, and Shimek have made comparative studies of the rate of evaporation in different habitats. Fuller most recently has studied the rates of evaporation in well understood stages of forest development which we discussed in the preceding paper. The sum total of evidence at hand indicates that the laws of succession and the physical conditions on the one hand and *growth-form* and *mores* on the other *are very generally in accord.*

Ecological classification of animals must be based upon community of physiological makeup, behavior, and mode of life. Those natural groups of animals which possess likenesses are the communities which we must recognize. One community ends and another begins where we find a general more or less striking difference in the larger *mores* characters of the organisms concerned.

7. *Ecological Terminology.*

Terminology in ecology is still unsettled and changing. Groupings have thus far been based upon similarity of habitat. Habitat likenesses have, in general, been based upon general resemblances. General resemblances have not always been accompanied by similar physical conditions, as was pointed out in the preceding paper of this series. In general, there has been an agreement in the recognition of strata, of associations as communities based upon the minor differences in habitat, and formations based upon the larger major differences in habitats. Dahl ('08) uses the term zoötope for formation and biocönose for association and apparently stratum also. Clements uses consocies for a division of a community dominated by some one species of plant; the term in this sense is less applicable to animals than to plants.

We give the communities of different orders below with taxonomic divisions of corresponding magnitude opposite, for comparison. With the exception of the first, these taxonomic groupings do not bear the slightest relation to the ecological groupings, but are added to indicate magnitude.

Mores (the term applied to animals possessing certain attributes) are groups of organisms in full agreement as to physio-

Dahl ('08)	Plant and Animal Ecologists	Taxonomic Groups.
Form	(Mos) mores (Shelford, '11)	Form (forms) (Species).
	Consocium (Clements, '06)	Genus.
Biocönose	Stratum or Story (Warning)	Family.
	Association or Society (Warning)	Order.
Zootope	Formation (Grisebach, '48; fide Clements).	Class.
	Extensive or Climatic Formations	Phylum.
	(Aquatic and Terrestrial.)	Vertebrates and Invertebrates.

logical life histories shown by the details of habitat preference, time of reproduction, reactions to physical factors of the environment, etc. The organisms constituting a *mores* usually belong to a single species but may include *more* than one species as *specificities of behavior* are not primarily significant (see p. 338).

Consocies are groups of *mores* usually dominated by one or two of the *mores* concerned and in agreement as to the main features of habitat preference, reaction to physical factors, time of reproduction, etc. Example: the prairie aphid consocium; the aphids control a group of organisms which for the most part prey upon them, as for instance, certain species of lace-wings, lady beetles, syrphus flies, etc.

Strata are groups of *consocies* occupying the recognizable vertical divisions of a uniform area. Strata are in agreement as to materials for abode and general physical conditions, but in less detail than the consocies which constitute them. (For differences of physical conditions see Table V., p. 84; Shelford, '12). For example, the beech forest animal community is clearly divisible into the subterranean-ground stratum, field stratum (level of the tops of the herbaceous vegetation), the shrub stratum (level of the tops of the dominant shrubs), the lower tree stratum (level of the shaded branches of the trees), and the upper tree stratum. A given animal is classified primarily, with the stratum in which it breeds, as being most important to it, and secondarily with the stratum in which it feeds, etc., as in many cases most important to other animals. The migration of animals from one stratum to another makes the division lines difficult to draw in some cases. Still the recognition of strata is essential though a rigid classification is undesirable. Consocies boring into wood of living trees, probably, should be considered as consocies relatively independent of stratification phenomena.

Associations are groups of strata uniform over a considerable area. The majority of *mores*, *consocies*, and *strata*, are different in different associations. A minority of strata may be similar though rarely identical. The unity of associations is dependent upon the migration of the same individual and the same *mores* from one stratum to another at different times of day or at different periods of their life histories. Such migration is far less frequent than from one association to another.

Formations are groups of associations. Formations differ from one another in all the strata, no two being closely similar. The number of species common to two formations is usually small (*e. g.*, 5 per cent.). Migrations of individuals from one formation to another are relatively rare.

To illustrate associations and formations, we have noted two great groups in the forest development series discussed. These groups are the cottonwood, pine, blackoak associations belonging to the sand area and in disagreement in the majority of *mores*, consocies, and strata; and the redoak, hickory, and beech associations belonging to the climatic forest proper and comparable with the first group in disagreement. The *mores* of the former are characteristic of sand areas, within the range of the deciduous forest climate. On this basis we may designate this as the *sand area animal formation of the deciduous forest climate* (Gleason, '10). It is here made up of the three *associations* just mentioned. The three later stages constitute the *deciduous forest animal formation*, which is here also made up of the three animal *associations*, named above. The two formations are separated upon the basis of striking differences in modes of life of the animals of the sand-dominated and forest-dominated communities and are in general disagreement as to *mores*, consocies, and strata concerned, only about 5 per cent. of the animals of the two hundred species listed in the preceding paper occurring sparingly or occasionally in the more similar associations of the two formations. The character of these differences was briefly outlined on pages 89–90 of the same paper (Shelford, '12[1]).

Extensive or climatic formations are groups of formations including all clearly influenced by a given climate in the case of land formations and (if recognizable in fresh water) by *topographic age* of a large area and by climate in the case of aquatic formations. For example, all of the thicket and other early stages of forest development of the area dominated by the deciduous forest formation constitute the deciduous forest extensive formation. Such formations occupy large areas which may be termed *ecological provinces* (Gleason, '10).

8. *Animal Communities in the Forest Border Region.*

The forest border region is the western line of demarkation of the deciduous forest climate. The following is a list of some animal communities about the south end of Lake Michigan. It is not intended to be complete, but rather to illustrate the use of the terms with particular reference to the communities mentioned in this series of papers.

I. Stream Communities.
    A. Intermittent Stream Communities.
        1. Horned Dace or Pool Association.
    B. Permanent Stream Communities.
        1. *Hydropsyche* or Riffle Formation.
        2. *Anodontoides ferussacianus*—Sand or Gravel Bottom Formations.
        3. Baselevel or Sluggish Stream Communities.
            a. Pelagic Formation.
            b. *Hexagenia lineata* or Silt Bottom Formation.
            c. *Planorbis bicarinatus* or Vegetation Formation.
II. Lake-Pond Communities.
    A. Pelagic Formations.
    B. *Pleurocera subulare* or Terrigenous Bottom Formation.
    C. Vegetation Formation.
        1. *Leptocerinæ* or Submerged Vegetation Association.
        2. *Neuronia* or Emerging Vegetation Association.
    D. Temporary Pond Formation.
III. Prairie or Grassland Formation of the Savanna Climate.
    A. Grassland Associations of Moist and Marshy Soil in the Savanna and Forest Climates.
    B. Prairie Chicken or Prairie Associations of the Savanna Climate.

IV. Thicket or Forest Margin Formations of the Savanna and Forest Climate.
    A. Wet Ground Thicket Associations. (Lower strata occasionally submerged.)
        1. River Deposit (Silt) or Stream Margin Thicket Sub-formations.[1] (First stage in the development of Flood Plain Forest.)
        2. Marsh and Pond Margin Thicket Sub-formation. (First association in the development of forest in marshes.)
        3. Candle-head or Moist Forest Margin or Thicket Sub-formation of the Savanna and Deciduous Forest Climates.
    B. *Straussia longipennis* of High Forest Margin Associations of the Savanna Climate. (A climatic association of considerable permanency.)

V. Forest Communities of the Deciduous Forest Climate.
    A. Formations on Sand in the Deciduous Forest Climate or Province.
        1. *Cicindela lepida* or Cottonwood Association.
        2. *Cicindela Lecontei* or the Pine Association.
        3. Ant Lion or Black Oak Association.
    B. Climatic Forest Formation of the Deciduous Forest Climate.
        1. Blackoak-Redoak Association.
        2. *Cicindela sexguttata* or Redoak-Hickory Association. (1 and 2 were treated together in the preceding paper and in the discussion above but may readily be separated.)
        3. *Rana sylvatica* or Beech-Maple Association.

Each division made here is based upon observations on the ecology of the animals constituting it. Marked differences in mode of life, reaction to physical factors, time of seasonal appearance, are noticeable between the formations and lesser differences of the same sort between the associations.

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[1] The term *association* is applied mainly to stages in the development of climatic and of old topography formations; *subformation* (Warning), to communities comparably different physiologically but not clearly genetically related, or to associations when no genetic relationships are implied. Thus here in a classification intended to be primarily physiological, the stream margin thicket is placed among the thicket formations while in a purely genetic classification as shown in the chief features of arrangement in Fig. 11 it would be termed a stage or association in flood plain forest development.

We note from Fig. 11 showing the relation of communities, that intermittent streams become permanent, gravel bottom gains ascendency over riffles and silt bottom over gravel bottom, which is accompanied by a decrease in strength of current. All types of streams converge to the base level stream, all large lakes toward small lakes, which are parallel with the sluggish streams. These small lakes become ponds and finally dry land, in the forest border region, either prairie or forest. Forest margin or thicket is a tension line and may shift position rapidly.

The relation of the different habitats is a genetic one, the most permanent habitats being the sluggish stream and climax plant communities. Each is characterized by *different mores*, and as the one habitat is transformed into another the *mores change accordingly*.

III. General Discussion.

The environmental processes, which we are discussing are those in which organisms have existed since their origin on earth. The stresses and strains to which organisms have been subjected have been in the same direction for long periods. Now that we have learned much concerning organic response to environment, such as physiological response, behavior response, and structural response, we note at once that processes of adjustment and equilibration of living substance may bear important relations, on the one hand to environmental processes and on the other to the physiological aspect of biological phenomena. Ecological classification is then worthy of attention.

With all of their imperfections and uncertainties, the ideas of phylogeny which are presented in our phylogenetic system of taxonomy are an important asset in zoölogical thinking from the point of view of structure and development. The classification which ecologists are striving to build up will serve a purpose in behavior, physiology, and ecology, analogous in this respect to that served by the phylogenetic classification in morphological thought. It should however be flexible rather than rigid and

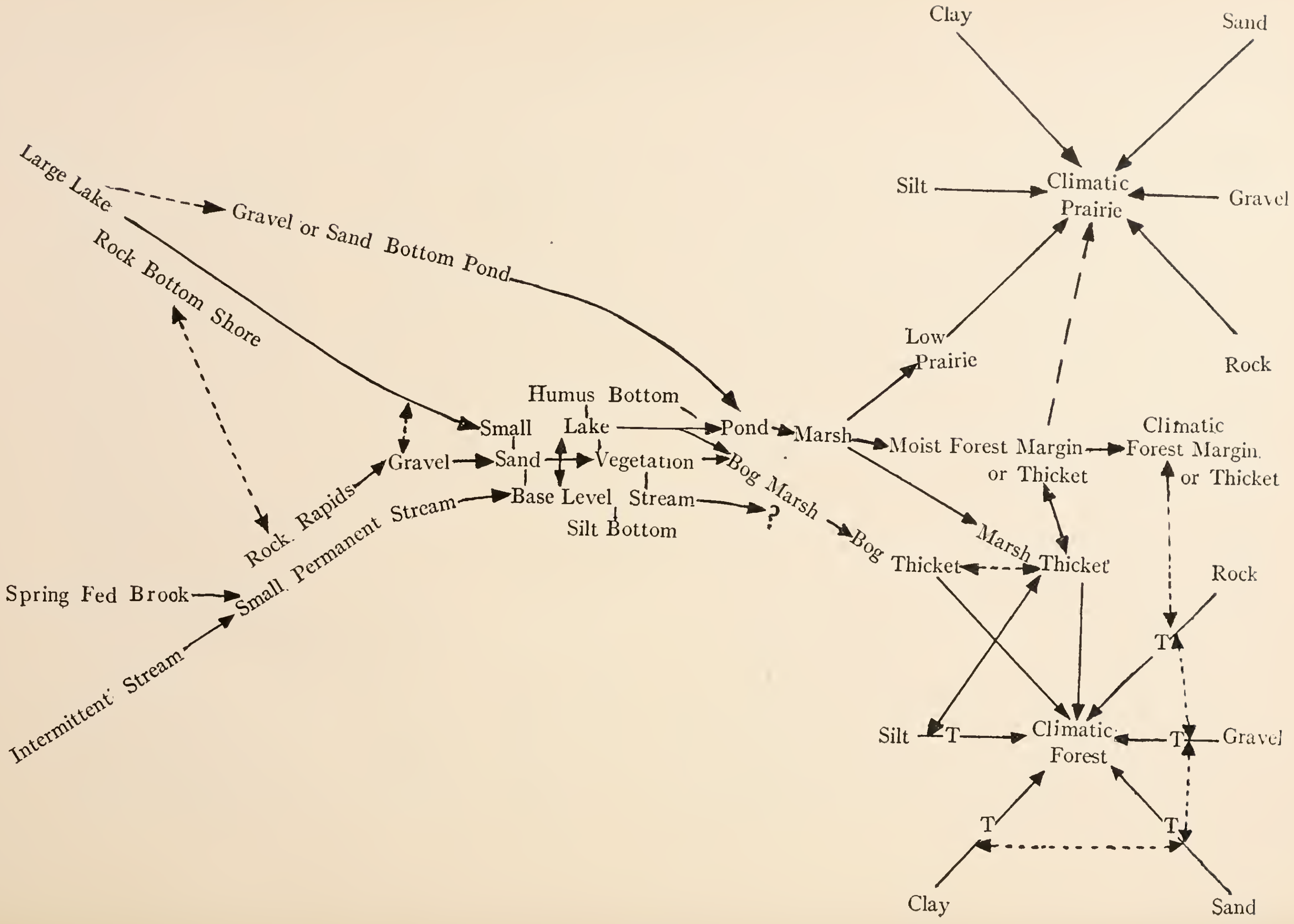
Clay
Sand
Silt
Climatic Prairie
Gravel
Large Lake
Gravel or Sand Bottom Pond
Rock Bottom Shore
Low Prairie
Rock
Humus Bottom
Small
Lake
Pond
Marsh
Moist Forest Margin or Thicket
Climatic Forest Margin or Thicket
Gravel
Sand
Vegetation
Bog Marsh
Base Level
Stream
Silt Bottom
?
Rock Rapids
Small Permanent Stream
Marsh
Bog
Thicket
Thicket
Rock
Spring Fed Brook
Intermittent Stream
T
Silt
T
Climatic Forest
T
Gravel
T
T
Clay
Sand

Fig. 11.

FIG. 11. Showing some relations of the chief animal communities of the forest border region of central North America. The word community or communities is to be understood as following all of the words or phrases appearing in the diagram. Single pointed arrows show the directions of succession, double pointed arrows show similarities of conditions and the occurrence of several or many of the same species in considerable numbers in some of the strata of communities between which such arrows extend. Broken lines indicate less definite relations than the solid lines. Starting with the aquatic communities we note that spring fed and intermittent stream communities converge with physiographic ageing to small permanent swift stream communities and permanent swift stream communities are succeeded by base level stream communities. The characteristic communities of small permanent streams and base level streams are indicated above them. Taking up another line we note that the large lake communities are succeeded by the small lake communities. Rocky shore communities of the large lake have features in common with those of the rocky rapids of the stream. The sand, gravel and vegetation communities of the baselevel stream and the small lake have many things in common, while the silt and humus bottom communities are distinguishing features of the two. Communities of ponds originating by very rapid physiographic changes pass through a series of stages comparable to those found in the different parts of the small lake. The lake communities pass to the pond community stage or give rise to a floating bog marsh community which is displaced by a floating bog thicket community. Cowles states that this takes place in deep lakes while the shallow ones become ponds which give rise to marshes with firm substratum; the marsh community may be displaced wholly by a low prairie community, in part by a thicket or forest margin community or wholly by a thicket community which will be succeeded by a forest community. In the savanna or prairie climate the marsh margin thicket may become a climatic thicket or forest margin. In the savanna or prairie climate the communities of all the various soils and the low prairie community may converge to the climate prairie community, or to the forest community as is shown below for the forest climate. In the forest climate and locally in the savanna climate the communities of all the various soils pass through a thicket community stage (*T*) related to a climatic forest margin. The thicket communities of all the dry soils are related to the forest margin or thicket community of the savanna climate.

true to fact rather than to schemes. Figuratively speaking, an ecological classification cuts taxonomy vertically, showing many structural adaptations as matters of stratum. It also cuts it again horizontally, showing ecological similarity in organisms, structurally and phylogenetically diverse. It therefore provides a new and different means of organization of data.

What is the significance in the fact (Shelford, '12[1]) that *Cicindela lepida* belongs to the ecological group, the cottonwood association, which we may say corresponds to an order, and to the subterranean ground stratum (corresponding to family) and to the *Cicindela lepida mores?* Furthermore that *Cicindela lecontei* and *Cicindela sexguttata* belong to respective different and older situations or associations? We note that the habitats in which the species occur are characterized by distinctly different soils, moisture, amounts of shade and light. We note furthermore that these animals are possessed of unusual powers of flight and are able to *select* conditions suited to their physiological constitution. Their *mores* characters are definite characters, which can be measured in terms of reactions to measured complexes of physical and other environmental factors. They are as clearly defined as any morphological taxonomic characters and can be measured with the accuracy of physical phenomena.

Doubtless to the student of genetics, the question of the origin of such characters and their fixation in heredity is a leading question. At this point we know little or nothing. Since nearly all species have definite habitat preferences and since many varieties differ slightly from the related species form in the matter of habitat preference, it is probable that origin of a slight change in habitat preference, meaning a slight change in *reaction* to *physical factors*, a *change in ecological optimum*, is usually an early correlative of the origin of new races. Still the so-called taxonomic characters may remain apparently unchanged while marked changes in habitat preference and in reaction to physical factors are being brought about in plastic animals (Allee, '12, p. 341). On the other hand, the segregation in the so-called pure lines and races, accomplished in experimental breeding, often appears to take place without any regard to environment (Cockerell, '08, p. 547). These two facts accepted as they stand are in full accord and we might conclude that there are no relations between primary ecological characters and taxonomic characters. Such however can hardly be strictly true, but we cannot see what the real relations may be. If our point of view of ecology is correct the *ecological* characters of a race experimentally segregated, or experimentally produced must in practice consist *primarily* of *reaction to physical factors* or *combinations of physical factors* or to entire environmental complexes, secondly of a definite rate of metabolism, time of appearance or the like, thirdly of specificity of behavior, and fourthly of structural characters modifying behavior (see p. 339, order that of expediency). Relatively fixed taxonomic integumentary characters have no bearing on ecological matters, not even according to the broadest definitions of the subject. The characters which are not related to the environment, and which are of no ecological value, are the ones quite generally used in breeding work, specificity of behavior standing second, and plastic structure third, *primary ecological matters usually receiving no adequate attention or only such attention as comes incidentally with the handling of the material;* the results consisting of noted differences in reaction to light of doubtful intensity and quality, or similar temperature differences, etc. The testing of primary ecological characters can be adequately conducted in three ways. First, by the measurement of reaction to all or several of the chief environmental factors under rigidly controlled conditions, with each factor accurately measured qualitatively and quantitatively, and with the measurements of the speed or intensity of the reaction repeatedly determined. Second, by testing the reaction of the animals to a graded environmental complex of known constitution, and third, by putting the animals out into a graded series of natural environments selected with due reference to the species in question. Being easily open to experiment, the question of the relation of taxonomic and ecological characters should be left for experimental studies to answer.

The relation of habitat preference to the so-called structural adaptations and to their origin is, as we have seen, not intimate, and the method of experimental attack less obvious than in the case above. In 1907 we attempted to point out possible relations of succession and isolation, to adaptations to strata.[1]

Turning to the tiger beetles to illustrate a mode of analysis of adaptation characters, we note that the entire family of Cicindelidæ is characterized by the same general type of mouth parts, same type of larvæ (R. Shelford, '07; V. E. Shelford, '08). Ovipositors, feet, and larval structures are somewhat different in the arboreal and terrestrial forms. The arboreal mode of life occurs at least so far as the adults are concerned, to a greater or less extent in each of the great tribes (Horn, '08, '10). The genera *Collyris* (Horn, '08, p. 99), *Pogonostoma* (*l. c.*, p. 86), and *Ctenostoma* (*l. c.*, p. 89) are quite generally arboreal (Horn, '08, '10). While the mode of origin of existing arboreal habits must remain a matter of conjecture from which we cannot hope to eliminate elements of subjective fancy, and while it is probable that representatives of arboreal groups have become terrestrial and vice versa, still the ground inhabitants are by far the most numerous and most like other Coleoptera. The differences between ground forms and ectophytic forms are clearly *more elementary* than such characters as mouth parts, general larval or ovipositor characters, because the former consist of *minor modifications* of these general characters. If the problem of adaptation may be attacked directly at all, we must first separate the *smaller* from the *larger adaptation* characters. This accomplished we must note the kinds of conditions to which the adaptation characters are related. In the case of the tiger beetles, as will be found to be true in many other cases, the more *elementary characters are adaptations to stratum.*

As succession proceeds, as we have noted in the preceding paper ('12[1]), conditions become progressively less favorable on the ground, for many animals, and the terrestrial members of the various groups give way to ectophytic forms of higher and higher levels. We have already noted that the process of peneplanation in the deciduous forest climate causes isolation of uplands with oak hickory forest, which finally give way to beech and maple (Cowles' unpublished observation). Thus the organisms of such a habitat are subject to increasingly greater degrees of moisture, denser shade, and different materials for abode, including the general absence of mineral soil; in fact all of the factors that are supposed to influence the course and origin of structural characters. The changes brought about by succession, as when the beech forest displaces the oak, are disadvantageous to such tiger beetles as *Cicindela sexguttata* because of the practical disappearance of mineral soil, and the movement of food species from the ground to the vegetation. Experimental conditions could easily be devised which would duplicate and intensify the changes alluded to, while acting upon some favorable organism. If new forms appeared under the experimental conditions any of them *selected a higher level on the plants of the experimental conditions, and possessed any structural characters which enabled them to succeed there*, we would have a case of *true adaptation paralleling the commonest type in nature*. It should be noted also that the fact that elementary adaptations are so often adaptations to stratum speaks in favor of that view of the origin of adaptations advanced by Eigenmann ('08) (selection of suitable habitat by animals possessing adaptation characters). The chief objection to this view seems to have been that animals could not in most cases reach a suitable habitat. We have noted that there are markedly graded (vertical differences) *stratification* conditions of light, temperature, circulation of medium and rate of evaporation. Movement of habitat preferences upward or downward is always a possibility readily attainable. Should an adaptation to a particular stratum become established, new lines of horizontal expansion would be thrown open. Such horizontal extension of range would not usually be accompanied by structural adaptations.

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[1] Address before the American Society of Zoölogists, December, 1907.

We have doubtless proceeded far enough with the statement of general ecological problems, to note that the training of the ecologist must at present be broad. In the near future, he must specialize upon some aspect of the subject, because it is *unusually large and its concepts especially complex*. At present he is called upon to know general zoölogy, especially general physiology and behavior of organisms. He must have a working knowledge of physiography, climatology, and plant ecology and must be able to analyze, or at least to understand both physical and chemical analysis of soil, water, and air, and be able to experimentally control the factors involved in these. To understand technical ecological work, one must be in very close touch with these fields as well as with special ecological matter. The complexity of the problems involved and the lack of training of zoölogists along these lines, is sufficient reason for the attitude of an occasional zoölogist toward the subject before its problems were clearly formulated.

IV. Summary.

1. Ecological or physiological classification of animals is *based* upon similarities and differences in *physiological life histories, reactions to physical factors* and the general physiology of environic relations, pp. 339, 354.

2. There is an agreement between *mores* of a community due to (*a*) *selection* of habitat through innate characters and (*b*) *modification* of behavior, p. 336.

3. The commonly recognized specificities of behavior are of little significance in ecological classification, p. 338.

4. Adaptation is of questionable significance in ecology; the most common adaptations are to strata or mode of food getting, p. 340.

5. Animal communities of greater magnitude are made up of those of lesser magnitude. The physiological agreement in those of greater magnitude is less close than in those of lower, pp. 354–56.

6. Animal communities are physiologically and genetically (succession) related and their genesis is determined by the genesis of the environment which is usually orthogenetic and converging, p. 359.

7. The relations of ecology to the phenomena of genetics and of adaptation, are not clear; no relations are apparent but actual relations are experimentally determinable.

Hull Zoölogical Laboratories.
University of Chicago,
August 25, 1912.

V. Acknowledgments and Bibliography.

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AN EXPERIMENTAL INVESTIGATION OF AN APPARENT REVERSAL OF THE RESPONSES TO LIGHT OF THE ROACH (PERIPLANETA ORIENTALIS L.).

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Roaches are nocturnal animals, they shun the light and seek the darkness. This has been well known for years; according to Szymanski, Graber[1] is to be credited with thoroughly demonstrating this. According to my observations, it would be incorrect to call this a case of negative phototropism, if we use the term in the sense that Loeb uses it; for there is no orientation to the rays of light, but simply a scampering hither and thither until some dark hole or crevice is found, into which the roach immediately rushes. Normally these roaches are exceedingly shy and any attempt to touch or handle them is responded to by suddenly darting away. Even the slightest touch is sufficient to cause them to run away. In these experiments an attempt has been made to apply the electrical punishment method devised by Yerkes[2] when experimenting with white mice to a study of the light reactions of one of the common roaches.

HISTORICAL RÉSUMÉ.

To the best of my knowledge, this method has been used only once in the study of insect behavior; that was by Szymanski,[3] who has recently made such a study of ten larval cockroaches of the species *Periplaneta orientalis* L. He studied ten male roaches all of the same age. Based on their ability to learn and upon evidences of fatigue, Szymanski classifies roaches as follows; those that with practice make rapid progress and fatigue slowly, those that with practice make rapid progress and fatigue rapidly, those that with practice make slow progress and fatigue rapidly. The average number of shocks required to induce a roach to make ten successive refusals to enter the dark chamber was 51, the least 16 and the greatest 118. "Marked individual differences were noted with respect to the time during which the cockroaches retained their newly acquired habit. . . . No relation is evident between the degree of permanency of the newly acquired habit and the number of shocks necessary to establish it." He gives learning curves, each of which he considers a special case of Kraepelin's "Arbeitscurve." He found that animals with amputated antennæ can learn; but, in the case reported, it required 126 shocks to induce it to make ten successive refusals to enter the dark chamber.

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Apparatus and Material.

The subjects of these experiments were the following types of the common cockroach (*Periplaneta orientalis* L.): adult females, adult males, larval females one half of an inch long, larval females one fourth of an inch long, adult females with amputated antennæ.

The following apparatus was used: an electric shocking platform, electric batteries, an induction coil, an electrical switch key, and discrimination boxes. Except for certain minor details of construction, the electrical shocking platform is identical with the one used by Szymanski. As used by me the platform consisted of a thick block of wood 28.5 centimeters long and 23.5 centimeters wide, on the top of which two flat copper forks were securely fastened with their tines interdigitating. Each fork had sixteen tines. These tines were 19 centimeters long and 0.8 centimeter wide and each was separated from its neighbors, on all sides, by a space about one millimeter wide. By means of a binding post and wire the handle of each of these forks was attached to one of the terminals of the induction coil, one to each terminal. The induction coil had once been part of a medical battery; the intensity of the shock was regulated by means of a sliding core. Between the battery cells and the induction coil there was a key for making and breaking the circuit.

Three kinds of glass discrimination boxes were used. Box number one was 25 centimeters long, 8 centimeters wide and 8 centimeters deep. By means of transverse glass partitions, this box was divided into three compartments. One partition was stationary, shutting off an end compartment 15 centimeters long. The other partition was adjustable, thus making it possible to vary the lengths of the other two divisions. In these experiments the middle compartment was usually a little less than 4 centimeters long, thus making the other end compartment a little more than 15 centimeters in length. The middle compartment communicated with each of the others by doors which faced each other. Each door was a square 2.5 centimeters long. By means of a special hood and an opaque screen the shape of the transverse partition, one of the end compartments was transformed into a dark chamber. The animal forming the subject of the experiment was placed in that end of the narrow middle compartment which was most remote from the exits. When the animal reached the opposite end of the narrow passage it had an opportunity to select which of the doors it would enter.

After reading Szymanski's paper, which appeared soon after I had begun my experiments, I decided to use a box similar to his. This decision was reached, not because the box described in the above paragraph proved unsatisfactory, but because, on account of the greater freedom, it took longer to perform an experiment with the box I designed than with the one designed by Szymanski. The fact that box number one permitted a roach in one compartment to select which of two others it would enter while Szymanski's box simply permitted it to enter the one towards which it was moving or else remain in the one where it was caused me, at first, to consider this box superior to Szymanski's; but, after much thought, it was decided that box number one was not sufficiently superior to Szymanski's to offset the advantage of shortening the time necessary for conducting an experiment. The shorter the time required for an experiment the less fatigue interferes with the reactions. Then, too, there are other reasons for desiring to shorten the time of intimate contact with these stench-engendering creatures. Box number two was 30 centimeters long, 3 centimeters wide and 8 centimeters high. One end was transformed into a dark chamber 19.5 centimeters long. Between the dark chamber and the lighted portion an opaque curtain dropped to within 2 centimeters of the floor.

Table I.

Showing the Ability of Adult Female Roaches to Learn to Avoid Entering a Specific Dark Place.

Roach No. 1. An old female that was the subject of these experiments for over thirty-six days. During that time she laid four oöthecæ. During the last three days she was quite feeble. She died soon after the close of the experiments of series *O*.

Successive Refusals to Enter the Dark Chamber.	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*K*	*L*	*M*	*N*	*O*	Series of experiments.
		8	14	4	21	24	504	24	4	120	72	27	40	24	Hrs. elapsed since close of last series.
I.	5	5	1	4	10	4	2	8	1	4	0	4	5	6	* Shock paralyzed the roach.
IV.	21	7	4	4	12	4	*	12	11	4	4	8	14	7	
VII.	21	7	4	*	18	9		12	11	4	43	8	16	14	† The roach became too feeble to react.
X.	21	7	5		18	9		12	11	4	43	11	18	†	

Roach No. 7. One of the quickest to learn of the adult females. These experiments extended over seven days.

Successive Refusals to Enter the Dark Chamber.	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	Series of experiments.
		24	24	24	24	24	24	24	Hrs. elapsed since close of last series of experiments.
I.	3	2	2	0	1	0	0	1	
IV.	3	2	2	0	1	0	2	2	
VII.	3	2	2	0	1	0	2	3	
X.	4	2	2	0	1	3	3	8	

Roach No. 8. One of the most retentive adult females. These experiments extended over eight days.

Successive Refusals to Enter the Dark Chamber.	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	Series of experiments.
		24	24	30	18	24	48	48	Hrs. elapsed since close of last series of experiments.
I.	4	0	1	2	0	2	5	12	While series *H* was being performed the roach was so weak that she could hardly walk.
IV.	4	0	2	2	0	2	5	25	
VII.	5	0	2	2	1	3	5	25	
X.	5	0	3	2	2	4	5	25	

To help interpret the behavior observed in the other two boxes, box number three was constructed. Like the others it was of glass. It was 25 centimeters long, 8 centimeters wide and 8 centimeters high. A dark chamber 15 centimeters long and of the same width and height as the glass box was placed in one end. In the middle of the partition which separated this dark chamber from the lighted portion of the glass box there was a door 3 centimeters wide and 2 centimeters high.

TABLE II.

SHOWING THE ABILITY OF ADULT MALE ROACHES TO LEARN TO AVOID ENTERING A SPECIFIC DARK PLACE.

<table>
<tr><td rowspan="2">Successive Refusals to Enter the Dark Chamber.</td><td colspan="6">Roach No. 15. This experiment extended over four days. The most apt and the most retentive of all of the adult males examined.</td></tr>
<tr><td><i>A</i></td><td><i>B</i>
24</td><td><i>C</i>
48</td><td><i>D</i>
24</td><td></td><td>Series of experiments.
Hrs. elapsed since the close of last series.</td></tr>
<tr><td>I.</td><td>2</td><td>0</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td>IV.</td><td>2</td><td>0</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td>VII.</td><td>3</td><td>0</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td>X.</td><td>3</td><td>0</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td></td><td colspan="6">Roach No. 18. This experiment extended over about ten days. One of the dullest males examined. See No. 19.</td></tr>
<tr><td></td><td><i>A</i></td><td><i>B</i>
48</td><td><i>C</i>
4</td><td><i>D</i>
24</td><td><i>E</i>
144</td><td>Series of experiments.
Hrs. elapsed since the close of last series.</td></tr>
<tr><td>I.</td><td>3</td><td>3</td><td>6</td><td>1</td><td>4</td><td></td></tr>
<tr><td>IV.</td><td>11</td><td>5</td><td>6</td><td>2</td><td>4</td><td></td></tr>
<tr><td>VII.</td><td>13</td><td>9</td><td>6</td><td>2</td><td>4</td><td></td></tr>
<tr><td>X.</td><td>16</td><td>9</td><td>10</td><td>4</td><td>5</td><td></td></tr>
<tr><td></td><td colspan="6">Roach No. 19. These experiments extended over about ten days. A very dull roach, one of the dullest males examined. See Ex. 18.</td></tr>
<tr><td></td><td><i>A</i></td><td><i>B</i>
48</td><td><i>C</i>
24</td><td><i>D</i>
144</td><td></td><td>Series of experiments.
Hrs. elapsed since the close of the last series.</td></tr>
<tr><td>I.</td><td>1</td><td>2</td><td>4</td><td>2</td><td></td><td></td></tr>
<tr><td>IV.</td><td>11</td><td>8</td><td>4</td><td>3</td><td></td><td></td></tr>
<tr><td>VII.</td><td>17</td><td>8</td><td>4</td><td>4</td><td></td><td></td></tr>
<tr><td>X.</td><td>17</td><td>11</td><td>4</td><td>4</td><td></td><td></td></tr>
<tr><td></td><td colspan="6">Roach No. 20. These experiments extended over about four days. One of the aptest males examined. See No. 19.</td></tr>
<tr><td></td><td><i>A</i></td><td><i>B</i>
48</td><td><i>C</i>
2</td><td><i>D</i>
24</td><td></td><td>Series of experiments.
Hrs. elapsed since close of the last series.</td></tr>
<tr><td>I.</td><td>3</td><td>4</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td>IV.</td><td>3</td><td>5</td><td>0</td><td>0</td><td></td><td></td></tr>
<tr><td>VII.</td><td>3</td><td>5</td><td>0</td><td>2</td><td></td><td></td></tr>
<tr><td>X.</td><td>3</td><td>7</td><td>6</td><td>4</td><td></td><td></td></tr>
</table>

DESCRIPTION OF THE EXPERIMENTS.

The roaches used in these experiments were kept, in solitary confinement, in jelly glasses. A piece of damp sponge supplied the necessary moisture and food was added from time to time. In some cases sand was placed in the bottom of the tumblers. As frequently as necessary the glasses were cleaned. When the time for the experiment arrived the roach was transferred from this jelly-glass to either the middle compartment of box number one or the lighted portion of box number two. If this was the roach's first experience in the box it immediately rushed into the dark chamber. The current was then turned on and kept on until the roach returned to the lighted portion of the apparatus, when it was immediately turned off. Sometimes the roach would rush back again, or even several times, into the dark chamber; but, usually, after receiving only one punishment, it would approach the dark chamber more cautiously than before, and if it entered at all did so very slowly, as though expecting something to happen. Sooner or later, on reaching the entrance to the dark chamber, the roach would pause, feel about with its antennæ, then turn about and walk away or else remain there and clean

TABLE III.

SHOWING THE ABILITY OF ONE-HALF INCH LARVAL FEMALE ROACHES TO LEARN TO AVOID ENTERING A SPECIFIC DARK PLACE.

Successive Refusals to Enter the Dark Chamber.	Roach No. 2. These experiments extended over thirty-seven days. The quickest to learn of the larvæ of this age.														
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*K*	*L*	*M*	*N*	*O*	Series of experiments.
		4	24	24	96	384	48	96	24	72	24	48	24	24	Hrs. elapsed since the close of last series.
I.	3	4	2	2	2	1	1	0	0	0	0	0	1	2	* Became paralyzed by the shock.
IV.	4	4	2	2	*	1	1	0	0	1	4	0	1	2	
VII.	4	*	2	2		1	1	0	3	1	4	8	2	2	
X.	4		2	2		1	3	0	3	1	4	8	2	2	
	Roach No. 3. These experiments extended over about forty-six days. Slow to learn, but the most retentive roach of this age.														
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*K*	*L*	*M*	*N*	*O*	Series of experiments.
		20	24	240	24	144	72	24	48	24	24	48	24	144	Hrs. elapsed since close of last series.
I.	3	4	4	2	1	0	0	0	0	0	0	0	0	0	* Paralyzed by the shock.
IV.	7	4	4	2	*	2	0	2	2	0	0	0	0	4	
VII.	8	5	8	2		2	0	2	2	0	0	0	0	4	
X.	8	6	8	2		2	0	2	2	0	0	0	0	4	
	Roach No. 4. These experiments extended over about twenty-four days. In aptness and retentiveness an average roach of this age.														
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*K*	*L*	*M*	*N*	*O*	Series of experiments.
		26	22	96	16	72	24	24	24	24	24	48	24	144	Hrs. elapsed since the close of last series.
I.	3	0	0	5	2	1	1	1	2	0	1	2	1	1	
IV.	3	5	1	5	3	1	1	1	2	0	1	2	1	1	
VII.	5	5	1	5	3	1	1	1	4	3	4	2	1	8	
X.	5	5	1	5	3	1	1	1	4	3	4	2	1	8	

Table IV.

Showing the Ability of One-fourth Inch Larval Female Roaches to Learn to Avoid Entering a Specific Dark Place.

Roach No. 39. These experiments extended over eight days. Slow to learn, but very retentive.

Successive Refusals to Enter the Dark Chamber.	A	B	C	D	E	F	G	H	Series of experiments.
		24	24	24	24	24	24	48	Hrs. elapsed since the close of last series.
I.	7	3	1	0	0	0	0	0	
IV.	16	3	1	0	0	2	0	1	
VII.	16	3	1	1	1	2	0	1	
X.	16	3	2	1	1	2	0	1	

Roach No. 10. These experiments extended over fourteen days. Of medium aptness in learning, but not very retentive.

Successive Refusals to Enter the Dark Chamber.	A	B	C	D	E	F	G	H	I	K	Series of experiments.
		24	24	24	24	24	24	48	24	144	Hrs. elapsed since close of last series.
I.	2	3	1	2	0	2	2	4	2	2	
IV.	4	3	13	2	2	4	8	6	2	2	
VII.	8	3	13	3	2	5	8	8	2	2	
X.	8	3	13	7	2	5	8	8	2	2	

Roach No. 41. These experiments extended over eight days. Quick to learn and retentive.

Successive Refusals to Enter the Dark Chamber.	A	B	C	D	E	F	G	H	Series of experiments.
		24	24	24	24	24	24	48	Hrs. elapsed since close of the last series.
I.	1	2	1	2	1	0	0	0	
IV.	3	2	1	2	1	0	1	1	
VII.	5	3	1	2	1	0	1	1	
X.	5	3	1	2	1	0	1	1	

Roach No. 13. These experiments extended over fifteen days. Quick to learn, but not very retentive.

Successive Refusals to Enter the Dark Chamber.	A	B	C	D	E	F	G	H	I	K	Series of experiments.
		24	24	24	24	24	24	48	24	144	Hrs. elapsed since the close of the last series of experiments.
I.	1	3	7	2	1	2	2	0	2	4	
IV.	1	4	7	3	2	2	2	2	3	4	
VII.	3	4	7	3	2	2	2	2	3	12	
X.	6	4	7	3	2	2	2	2	3	12	

its antennæ and other appendages. Occasionally it would simply stand there and wave its antennæ. Whenever the roach did not, of its own accord, approach the dark chamber and whenever it paused for some time before the entrance to it, I stroked its back or even gently shoved it towards the darkness. The stroking and shoving was done with a piece of wire the end of which had been rounded to prevent scratching. Accurate records were kept of the behavior of each roach and of the number of shocks given. Whenever a roach made ten successive refusals to enter the dark chamber the experiment was terminated for that time. Except where I was testing the result of intervals of less than a day, the experiments were conducted at about the same time each day.

Some of the results of these experiments are recorded in the accompanying tables. Although the records of all of the experiments performed have not been tabulated, yet the selected experiments exhibit all of the types of behavior observed. The Arabic numerals in the narrow vertical columns indicate the number of shocks necessary to cause the individual to make the number of successive refusals to enter the dark chamber that is indicated by the Roman numeral in the column to the extreme left. Each shock represents an error made by the roach. For the sake of

Table V.

Showing the Ability of Adult Females with Amputated Antennæ to Learn to Avoid Entering a Specific Dark Place.

Successive Refusals to Enter the Dark Chamber.	Roach No. 61. These experiments extended over four days. An average antennaless female.				
	A	*B*	*C*		Series of experiments.
		24	48		Hrs. elapsed since the close of the last series.
I.	7	7	2		
IV.	18	7	2		
VII.	18	8	2		
X.	18	8	2		
	Roach No. 21. These experiments extended over ten days. The brightest antennaless female examined.				
	A	*B*	*C*	*D*	Series of experiments.
		48	24	144	Hrs. elapsed since the close of the last series.
I.	2	2	1	2	
IV.	3	4	5	3	
VII.	12	7	5	3	
X.	16	8	5	5	
	Roach No. 22. These experiments extended over four days. The dullest antennaless female examined.				
	A	*B*	*C*		Series of experiments.
		48	24		Hrs. elapsed since the close of the last series.
I.	2	1	2		
IV.	2	5	3		
VII.	24	11	3		
X.	26	16	3		

uniformity, all of the tabulations were made from experiments performed with box number two.

In every case, sooner or later, the roach always learned to avoid entering the dark chamber; and this was true whether I used discrimination box number one or discrimination box number two. With box number one, where greater freedom was allowed, it usually required more time to establish the habit. As a group adult male roaches learned to avoid the dark chamber more quickly than adult females and young females much more quickly than old females. The slowest of all of the roaches to learn were adult females with amputated antennæ (Fig. 1).

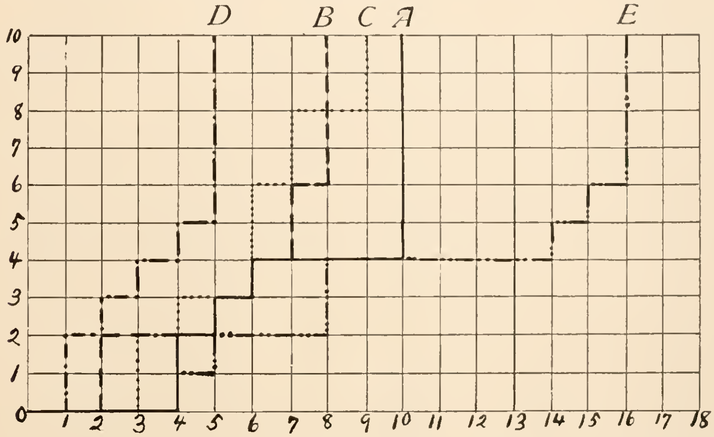

Fig. 1. Learning curves of roaches. Each of these curves represents the average of ten roaches of the kind indicated. The abscissas represent electric shocks, the ordinates the number of refusals to enter the dark chamber that were made before receiving the next shock. *A* represents adult female roaches; *B*, adult male roaches; *C*, larval females one half inch long; *D*, larval females one fourth of an inch long; *E*, adult females with amputated antennæ.

There is a marked contrast between the behavior of adult roaches with amputated antennæ and ordinary adults. The normal roach usually moves along the middle of the passageway until it reaches the entrance to the dark chamber, which it either enters or refuses to enter. Before receiving punishment these movements are rapid; after receiving one or more shocks, the roach moves along more slowly and more cautiously. If it approaches the sides at all it is for the purpose of attempting to climb up them to freedom. On the other hand, the roaches with amputated antennæ move along with a side of the head in contact with one of the side walls of the discrimination box; reminding one very much of a blind person groping along. Usually the movements are rapid, and it requires much punishment to cause the roach to avoid the dark chamber. Indeed, the whole behavior of these antennaless roaches impresses one with the thought that the antennæ play the same prominent rôle in the behavior of roaches that the eyes do in the behavior of man. Other senses are used, but the antennal sense seems to be the one upon which most reliance is placed.

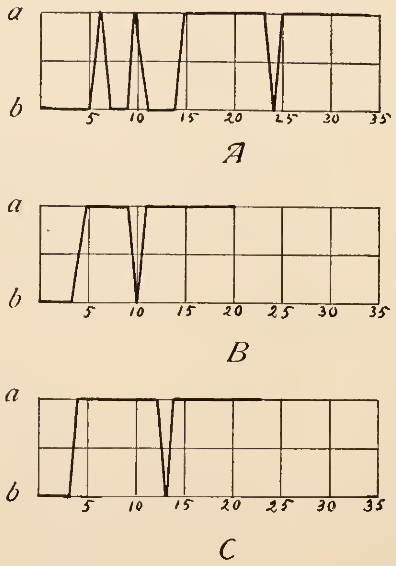

Fig. 2. Reaction curves of three adult females. The numbers represent opportunities to make a choice; *a* represents refusals to enter the dark chamber, *b* represents entrances into the dark chamber.

I stated above that male roaches are more apt than females and that young roaches are more apt than adults. Restricted to the average of each group this assertion is true; but, when we consider individuals as such, we can make no such universal statement. I have encountered males (Fig. 3, *E*) that were much slower to learn than dull females (Fig. 2, *A*) and I have seen larval females that were less apt (Fig. 4, *B*; Fig. 4, *D*) than adult females (Fig. 2, *A*). The quickest to learn of all of the roaches investigated was a male (Fig. 3, *C*). Ranking next to this male came an adult female with amputated antennæ (Fig. 6, *A*). Indeed the most striking thing in these investigations is the marked individuality of the roaches. A glance at the few reaction curves published herewith (Figs. 2–6) will serve to emphasize this statement.

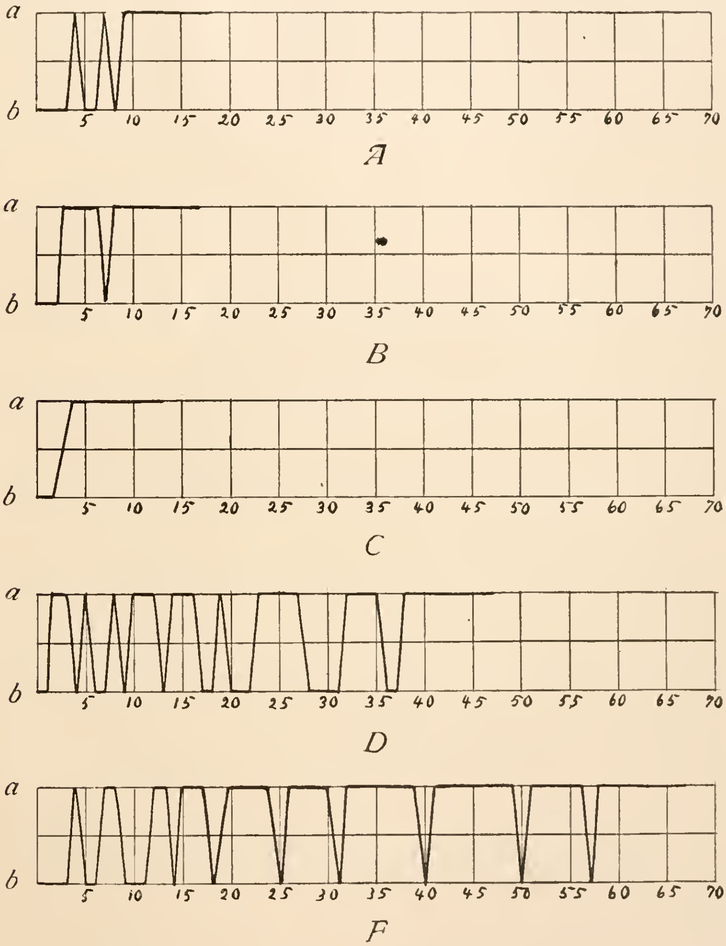

FIG. 3. Reaction curves of five adult male roaches. The numbers represent opportunities to make a choice; *a* represents refusals to enter the dark chamber, *b* represents entrances into the dark chamber.

Szymanski, in his study of larval male cockroaches, arranged them in three classes: those that learn rapidly and fatigue slowly, those that learn rapidly and fatigue rapidly, those that learn slowly and fatigue rapidly. Arbitrarily I can classify the roaches studied by me in the same manner; but, there are no sharp demarcating lines. It is also possible to divide roaches into groups based upon their ability to learn and to retain what they have acquired. Some roaches are quick to learn and retain well what they have acquired (Table I., numbers 7 and 8; Table II., numbers 15 and 20; Table III., number 2; Table IV., number 41); some are quick to learn but not very retentive (Table IV., number 13); some are slow to learn, but retain well what they

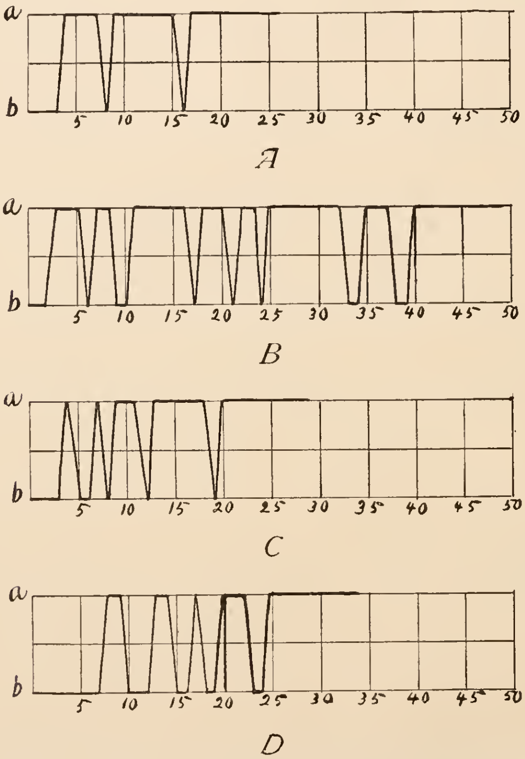

FIG. 4. Reaction curves of four larval females one half of an inch long; *a* represents refusals to enter the dark chamber, *b* represents entrances into the dark chamber.

have acquired (Table III., number 3, Table IV., number 39); some are slow to learn and not very retentive (Table I., number 1, Table II., numbers 18 and 19); some in learning display mediocre ability but retain well what they have acquired (Table III., number 4); yet others display mediocre ability to learn and are not very retentive (Table IV., number 41).

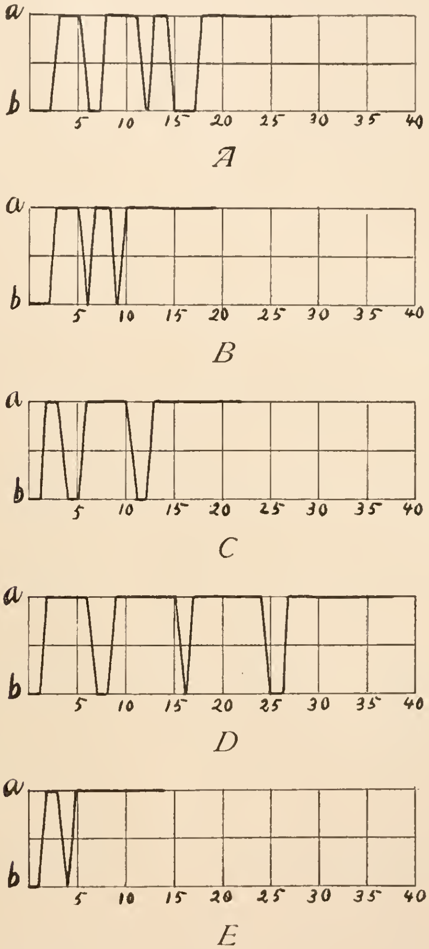

FIG. 5. Reaction curves of five larval females one fourth of an inch long; *a* represents refusals to enter the dark chamber, *b* represents entrances into the dark chamber.

Szymanski states that "No relation is evident between the degree of permanency of the newly acquired habit and the number of shocks necessary to establish it." With this statement my experiments are in accord.

The results of training persist for a long time. Unequivocal evidence of the persistence of the results of training were observed after the following intervals; one day or less (all of the tables), two days (Table II[1]., 15*C*, 18*B*, 19*B*, 20*B*; Table III., 2*G*, 2*M*, 3*I*, 3*M*), three days (Table III., 3*G*, 2*K*, 4*F*), four days (Table III., 2*E*), five days (Table I., 1*K*), six days (Table II., 18*E*, 19*D*; Table III., 3*F*, 3*O*), ten days (Table III., 3*D*), sixteen days (Table III., 2*F*), twenty-one days (Table I., 1*G*). Moult-

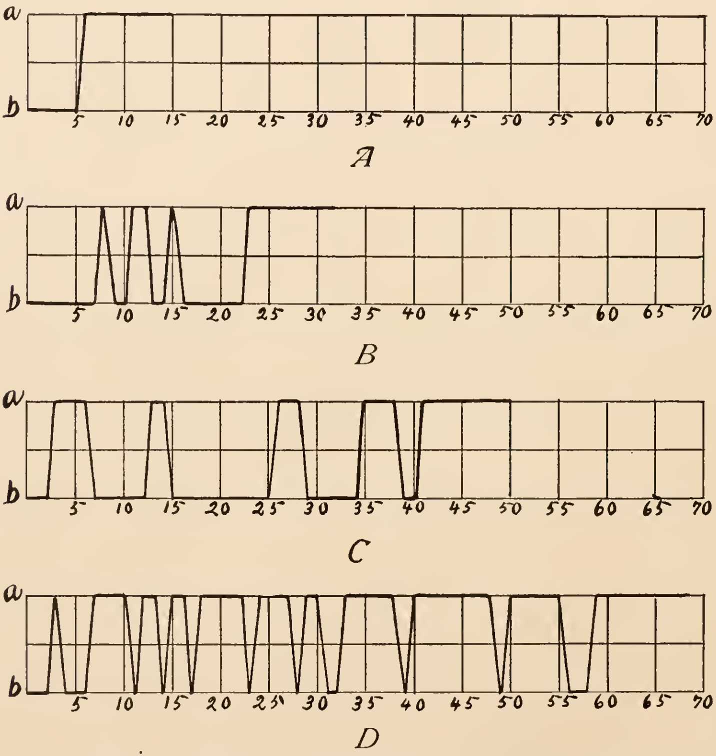

FIG. 6. Reaction curves of three adult females from which the antennæ have been amputated; *a* represents refusals to enter the dark chamber, *b* represents entrances into the dark chamber.

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[1] Throughout this section, the arabic numerals are the numbers of the roaches and the capital letters represent the series of experiments.

ing does not affect the retentiveness of larval roaches. Several of my larval roaches moulted during the progress of these experiments; but, except when the experiment was performed before the body had become sufficiently hard to permit freedom of movement, I never once detected any interference with the retentiveness of the roach. When the health of a roach is impaired and especially when it is dying, there is a marked falling off in its ability to retain the results of experience.

What is the meaning of this refusal of these roaches to enter the dark chamber? Can it be that a few electric shocks have produced such physiological changes in these insects that whereas once they reflexly sought the darkness now they reflexly shun it? Or, is it a case of having learned to avoid a particular dark place on account of certain unpleasant experiences? To find an answer to this question use was made of discrimination box number three. As has been stated above, this was a glass box 25 centimeters long, 8 centimeters wide and 8 centimeters high, in one end of which was a dark chamber 15 centimeters long. The lighted portion of the box communicated with the dark chamber by means of a door 3 centimeters wide and 2 centimeters high. Roaches that had thoroughly learned to avoid the dark chamber were tested in box number two and then transferred, at once, to the lighted portion of box number 3. Immediately such a roach would enter the dark chamber. It was then replaced in the lighted portion of box number 2, where it refused to enter the dark chamber and could not be induced to do so by the method mentioned above. Adult females, adult males, larval females one half inch long and larval females one fourth of an inch long were put through this test. With all such roaches that had thoroughly learned to avoid the dark chamber of discrimination box number two the responses were as stated. Roaches which had not thoroughly learned the refusal reaction and adult females with amputated antennæ, on being returned to the lighted portion of box number two, usually entered the dark chamber. To my mind this test is a conclusive proof that the change in the behavior of these insects is not due to a physiological reversal of the phototropic responses of the roaches; but a case of learning, by experience, to avoid a specific dark place because of certain disagreeable experiences connected with it.

Conclusions.

1. By means of electric shocks roaches can be trained to avoid entering a specific dark place. This is not a reversal of the phototropic responses of the roaches; but the result of learning to avoid a specific dark place because of certain disagreeable experiences associated with it.

2. Generally speaking male roaches learn more quickly than females and young roaches are more apt than adults; but there are marked individual exceptions to this.

3. In the ability to learn and to retain what they have acquired roaches exhibit marked individuality.

4. Roaches that have acquired the habit of refusing to enter a specific dark place do not lose that habit when they moult.

5. During sickness and just prior to death the retentiveness of the roach is much impaired.

September 20, 1912.

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